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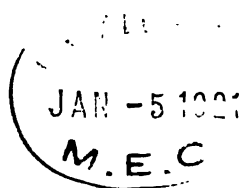
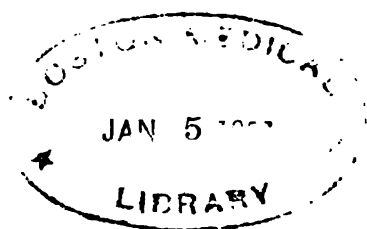
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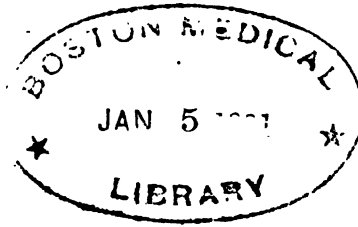
JOURNAL OF MORPHOLOGY, VOL. 34, NO. 1
JUNE, 1920

Resumen por la autora, Ethel Browne Harvey.
Princeton, New Jersey.

Una revisión del número de cromosomas en los Metazoos.
Parte II.

El presente trabajo es una lista del número de cromosomas de los anélidos, artrópodos y celentéreos, suplementaria a la lista publicada en la Parte I de este trabajo (Journ. Morph, v. 28, Diciembre 1916); en él se incluye además una lista completa del número de cromosomas en todos los invertebrados (excepto los Protozoos) y vertebrados hasta la fecha (Diciembre, 1918). Se incluye en el trabajo una corta introducción histórica y crítica. De los datos considerados se derivan las siguientes conclusiones: 1) Que el número de cromosomas en una misma especie es constante con algunas excepciones; 2) que existe un número definido de cromosomas característico de un grupo de animales próximos entre sí (número tipo); 3) que los cambios en el número de cromosomas de formas próximas se han originado mediante fisión o a consecuencia de la fusión de cromosomas. También se incluye en el trabajo una corta revisión de la presencia de heterocromosomas, incluyéndose así mismo una tabulación de la presencia de cromosomas sexuales.

Translation by José F. Nonides
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A REVIEW OF THE CHROMOSOME NUMBERS IN THE METAZOA

PART II

ETHEL BROWNE HARVEY

I. INTRODUCTION

The first part of 'A Review of the Chromosome Numbers in the Metazoa' appeared in 1916 in Volume 28 of this Journal.¹ This comprised a tabulation of all chromosome numbers in Annelida, Arthropoda and Coelenterata published until the end of 1915. The present paper includes a supplementary list of numbers in these three groups which have been published from the beginning of 1916 to date (Dec. 1918), and a complete tabulation of all the other Metazoa from 1878 to date. As in the previous paper, the species are classified and the various phyla, genera, families and species are arranged in alphabetical order. Access has been had in all cases to the original publications, and all authorities are quoted in order of priority, whether in agreement or otherwise. The list includes only normal animals; the chromosome numbers of hybrids and of abnormal and pathological cases are not included. Some of the most important abbreviations used are the following:

chrom = chromosome
cl = cleavage
-cyte = spermatocyte or oocyte
div = division
el = elements
emb = embryo
fert = fertilization
oog = oögonia

pa = parthenogenetic
p. b. = polar body
prim. = primordial
pron = pronucleus
som = somatic
spc = spermatocyte
spg = spermatogonia
-tid = spermatid or ootid

'X to pole' means that X passes undivided to one pole.

'XY to poles' means that X and Y pass to opposite poles.

'From correction in' means that the tabulation as given is a correction of an earlier account.

¹ I wish to call attention to a misplacement in Part I. On page 18, the group beginning with *Chrysochus auratus* and ending with *Trirhabda virgata* belongs with the Chrysomelidae on page 16 and not with the Hydrophilidae.

II. TABULATION OF CHROMOSOME NUMBERS

PART I. SUPPLEMENT (1915-1918)

SPECIES	DIPLOID AND PARHENO- GENETIC	1ST -CTYB	2ND -CTYB	-TID	OBSERVER	REFERENCE
A. INVERTEBRATA						
II. ARTHROPODA						
<i>b. Crustacea</i>						
<i>f. Malacostraca</i>						
<i>b. Decapoda</i>						
1. Brachyura (Part I, p. 14)						
Cancer magister.....	100 + spg	60♂	60♂		Faaten, '18	Biol. Bull., 34, p. 277
Chromatoid body in half of sperma- tids						
c. Insecta						
<i>f. Coleoptera</i>						
d. Chrysomelidae (Part I, p. 16; see foot-note 1)						
Diabrotica vittata.....	21♂ emb 22♀ emb	11♀		11♀ pron	Hoy, '18	Biol. Bull., 35, p. 166
<i>f. Coccinellidae (Part I, p. 17)</i>						
Epilachna borealis.....	18 cl			XX in ♀, XY in ♂	Hoy, '18	Biol. Bull., 35, p. 166
<i>g. Lucanidae</i>						
Passalus cornutus.....	26 spg 26♀ som	13♂		XY to poles in 1st	Schaffer, '17	Biol. Bull., 32, p. 407
g. Diptera						
a. Anthomyiidae (Part I, p. 21)						
Fucellia marina.....	12 som				Meta, '16	J. Exp. Zool., 21, p. 213
Homalomya sp.....	12 som		6♂		Meta, '16	J. Exp. Zool., 21, p. 213
Ophrya leucostoma.....	12 spg	6♂			Meta, '16	J. Exp. Zool., 21, p. 213

a³. Aelidae

<i>Ailus lecythus</i> }	14 spg	7♂	XY	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Ailus notatus</i> }					
<i>Ailus sericeus</i>	10 spg			Metz, '16	J. Exp. Zool., 21, p. 213
<i>Dasylla thoracica</i>	10 spg	5♂	XY	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Deromyia winthemi</i>	12 spg	6♂	XY	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Erax rufibarbis</i>	10 spg	5♂		Metz, '16	J. Exp. Zool., 21, p. 213
<i>Leptogaster badius</i>	10 spg	5♂	XY	Metz, '16	J. Exp. Zool., 21, p. 213

a⁴. Bombyliidae

<i>Anthrax lateralis</i>	12 spg			Metz, '16	J. Exp. Zool., 21, p. 213
<i>Anthrax sinuosa</i>	18 spg	9♂	XY	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Spogostylum simson</i>	12 oog		From figure	Metz, '16	J. Exp. Zool., 21, p. 213

d. Culicidae (Part I, p. 21)

<i>Culex pipiens</i>	6 spg 6 oog	3♂	3♂	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Culex pipiens</i>	6 spg 6 oog 6 som			Whiting, '17	J. Morph., 28, p. 523
<i>Culex pipiens</i>	6 som			Hance, '17	J. Morph., 28, p. 579
<i>Culex pipiens</i>	6 som.			Holt, '17	J. Morph., 29, p. 607
<i>Culex pipiens</i>	6 cl 3 (double) som			Taylor, '17	Q. J. M. S., 62, p. 287

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CTE	2ND -CTE	-TID	REMARKS	OBSERVER	REFERENCE
d ² . <i>Drosophilidae</i> (see under f. <i>Muscidae acalyptratae</i> , Part I, p. 22)							
<i>Cladochaeta nebulosa</i>	8 oog					Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila affinis</i>	10 spg 10 oog					Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila amoena</i>	8 spg 8 oog				XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila ampelophila</i> ..	8 oog				XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila ampelophila</i> ..	8 spg 8 oog				XY	Bridges, '16	Genetics, 1, p. 1
<i>Drosophila bromeliae</i>	8 spg					Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila busckii</i>	8 oog	4♂				Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila carlini</i>	12 oog					Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila caribae</i>	8 spg 8 oog				XY	Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila dimidiata</i>	8 oog					Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila earlei</i>	6 oog					Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila florae</i>	8 oog					Meta, '16	Amer. Nat., 50, p. 587
<i>Drosophila funebris</i>	12 spg 12 oog				Note i: '14 corrected	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila limbata</i> (ne- bulosa).....	8 oog					Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila melanica</i> (ne- glecta).....	10 spg 10 oog		5♂			Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 50, p. 587
<i>Drosophila modesta</i>	12					Meta, '16	Amer. Nat., 50, p. 587

<i>Drosophila obscura</i>	10 spg 10 oog	5♂		XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila ornateirennia</i> ...	11 oog			XY. One super- numerary	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila pallida</i>	8				Meta, '16	Amer. Nat., 80, p. 587
<i>Drosophila procerea</i>	8 oog				Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila ramadani</i>	12 oog			=Sp. A of '14	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila repleta</i>	12 spg 12 oog			XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila robusta</i>	8 spg				Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Drosophila saltana</i>	8				Meta, '16	Amer. Nat., 80, p. 587
<i>Drosophila similia</i>	12 oog				Meta, '16	Amer. Nat., 80, p. 587
<i>Drosophila tripunctata</i> ...	8 oog				Meta, '16	Amer. Nat., 80, p. 587
<i>Drosophila virilis</i>	12 oog			=Sp. B of '14	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Scaptomyza adusta</i>	10 oog			XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587
<i>Scaptomyza graminum</i> ...	8 spg 8 oog	4♂		XY	Meta, '16 Meta, '16	J. Exp. Zool., 21, p. 213 Amer. Nat., 80, p. 587

e. Muscidae (Part I, p. 22)

<i>Calliphora erythrocephala</i>	12 oog 12 som	6♂			Meta, '16	J. Exp. Zool., 21, p. 213
<i>Musca domestica</i>	12 oog				Meta, '16	J. Exp. Zool., 21, p. 213
<i>Phormia regina</i>	12 spg	6♂		XY to poles in 1st	Meta, '16	J. Exp. Zool., 21, p. 213

e³. Ortalidae (see under f. *Musca scalyptrotatae*, Part I, p. 22)

<i>Camptoneura picta</i>	12 spg	6♂			Meta, '16	J. Exp. Zool., 21, p. 213
<i>Chaetopsis fulvifrons</i>	8 spg	4♂			Meta, '16	J. Exp. Zool., 21, p. 213

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
e ³ . Sapromyzidae							
<i>Physeggenia vittata</i>	12 spg					Metz, '16	J. Exp. Zool., 21, p. 213
g. Sarcophagidae (Part I, p. 23)							
<i>Ravinia peniculata</i>	12 oog					Metz, '16	J. Exp. Zool., 21, p. 213
<i>Sarcophaga tuberosa</i>	12 spg				XY to poles in 1st.	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Sarcophaga saraceniæ</i>	12 oog	6♂	6♂		Multiple, some groups with 24 and 48		
<i>Sarcophaga</i> sp.....	12 som						
g ⁴ . Sciomyzidae							
<i>Neuroctena analis</i>	12 spg		6♂		From figures	Metz, '16	J. Exp. Zool., 21, p. 213
g ⁵ . Sepsidae							
<i>Piophilæ casei</i>	12					Metz, '16	J. Exp. Zool., 21, p. 213
g ⁶ . Stationomyidae							
<i>Ptecticus trivittatus</i>	16				XY	Metz, '16	J. Exp. Zool., 21, p. 213
h. Syrphidae (Part I, p. 23)							
<i>Eristalis bastardi</i>	12 spg				XY	Metz, '16	J. Exp. Zool., 21, p. 213
<i>Mesogramma marginata</i>		6♂					
<i>Volucella obesa</i>							
h ¹ . Trypetidae							
<i>Euaeresta melanogaster</i> ...	12					Metz, '16	J. Exp. Zool., 21, p. 213

4. Hemiptera
a. Hemiptera heteroptera
1. Belostomatidae (Part I, p. 23)

Belostoma (Zaitiba) fluminea.....	24 spg	13♂	13♂	XY to poles in 2nd	Chickering, '16	Trans. Amer. Micr. Soc. 38, p. 45
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2. Coreidae (Part I, p. 24)

Anasa tristis.....	21 spg, 21♂ el 21♂ som 22 oeg, 22♀ el 22♀ som			Some cells investing ovary have 44	Hoy, '16	Biol. Bull. 31, p. 339
Leptocoris haematoloma.	13 spg	7♂	7♂	X to pole in 2nd	Porter, '17	Biol. Bull. 33, p. 316

3. Nepidae

Ranatra sp?.....	40 spg	21♂	20♂	20♂	XY to poles in 2nd. Another type has 8 or 10 more chroms in spg	Chickering, '18 Trans. Amer. Soc., 37, p. 132
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10. Notonectidae (Part I, p. 30)

Notonecta glauca.....	24 spg 24 oeg	13♂	12♂	12♂	XY to poles in 2nd	Browne, '16 Jour. Morph., 27, p. 119
Notonecta irrorata.....	28 spg 28 oeg	14♂	13♂	13♂	XY to poles in 2nd	Browne, '16 Jour. Morph., 27, p. 119
Notonecta indica.....					XY to poles in 2nd. Wrongly tabulated in Part I	Browne, '16 Jour. Morph., 27, p. 119
Notonecta shooteri.....						
Notonecta undulata.....						
Notonecta insulata.....						

14. Reduviidae (Part I, p. 35)

Pseuloides (milyas) cinctus.....	28 spg 30 oeg	16♂	16♂	XY; X=3 elements	Goldsmith, '16	Biol. Bull., 31, p. 121
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II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>6. Hymenoptera</i>							
b. Chalcidoidea (Part I, p. 43)							
<i>Paracopidosomopsis floridana</i>	8 spg 8♂ som		8♂	8♂	Pa. generation. 1st div. abortive	Patterson and Porter, '17	Biol. Bull., 33, p. 38
	16 ol 16 ♀ som	8 ♀	8 ♀		Fertilized egg	Patterson, '17	Biol. Bull., 33, p. 57
<i>7. Odonata</i> (See under Neuroptera, Part I, p. 51)							
<i>Anax junius</i>	27 spg					Smith, '16	Biol. Bull., 31, p. 269
<i>Libellula basalis</i>	25 spg	13♂	12, 13♂		X to pole in 1st	Smith, '16	Biol. Bull., 31, p. 26 ^c
<i>Sympetrum semicinctum</i>	25 spg	13♂	13♂		X to pole in 2nd	Smith, '16	Biol. Bull., 31, p. 26 ^f
<i>8. Orthoptera</i>							
a. Acrididae (Part I, p. 51)							
<i>Aceridium</i> , see under Tet- tigidae							
<i>Chloasitis</i>	17 spg					McClung, '17	Jour. Morph., 29, p. 519
<i>Chorthippus</i> (<i>Stenoboth- rus</i>) <i>curtipennis</i>	17 sp ^c	9♂	8, 9♂		X to pole in 1st	Robertson '16 Lewis and Robert- son, '16	Jour. Morph., 27, p. 179 Biol. Bull., 30, p. 99
<i>Chorthippus</i> (<i>Stenoboth- rus</i>) <i>curtipennis</i>		9♂			X	Wenrich, '17	Jour. Morph., 29, p. 471
<i>Circotettix lobatus</i>	21 spg	11♂	10, 11♂		X to pole in 1st. Supernumeraries (1 or 2) may be present, to pole in 1st	Carothers, '17	Jour. Morph., 28, p. 445
<i>Circotettix rabula</i>							
<i>Hesperotettix brevipen- nia</i>	23 spg	12♂				McClung, '17	Jour. Morph., 29, p. 519
<i>Hesperotettix festivus</i>							

<i>Hesperotettix pratensis</i> <i>Hesperotettix speciosus</i>	22 spg (=28) 22 ♀ som (=24)	11♂ (=12) 9-12♂ (=12 or 12+1 st.)	11♂ (=11, 12) 10-12♂ (=11, 12)	11♂ (=11, 12)	X attached to an- other chrom. to pole in 1st	McClung, '17	Jour. Morph., 29, p. 519
<i>Hesperotettix viridis</i>	19-22 spg (=23)				X attached or free to pole in 1st. Other chroms may be fused in pairs. Supernu- merary present in one animal	McClung, '17	Jour. Morph., 29, p. 519
<i>Mermeria bivittata</i>	22 spg (=23) 22 ♀ som (=24)	11♂ (=12)	11♂ (=11, 12)	11♂ (=11, 12)	X attached to an- other chrom. to pole in 1st. 1 ar- ranged account cor- rected	McClung, '17	Jour. Morph., 29, p. 519
<i>Mermeria</i> sp?.....	23 spg	12♂				McClung, '17	Jour. Morph., 29, p. 519
<i>Nomotettix</i> } see under <i>Paratettix</i> } Tettigidae							
<i>Phrynotettix magnus</i>	23 spg	12♂	11, 12♂	11, 12♂	X to pole in 1st	Wenrich, '16	Bull. Mus. Comp. Zool. Harvard, 66, p. 56
<i>Stenobothrus</i> , see <i>Chor-</i> <i>thippus</i>							
<i>Syrbula scuticornis</i>	23 spg	12♂	11, 12♂		X to pole in 1st	Robertson, '16	Jour. Morph., 27, p. 179
Tettigidae (Subfamily of Acrididae)							
<i>Acridium granulatius</i> ..	13 spg 13♂ som 14 ♀ som	7♂	6, 7♂		X to pole in 1st	Robertson, '16	Jour. Morph., 27, p. 179
<i>Acridium incurvatus</i> ...	13♂ som	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
<i>Acridium obscuro</i>	13 spg	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
<i>Acridium ornatus</i>		7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
<i>Nomotettix cristatus</i> ...	13 spg				X	Rayburn, '17	Kansas Univ. Sc. Bull. 10, p. 267
<i>Paratettix cuculatus</i> ...	13 spg	7♂			X	Robertson, '16	Jour. Morph., 27, p. 179
<i>Paratettix texanus</i>		7♂			X	Robertson, '16	Jour. Morph., 27, p. 179

II. ARTHROPODA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
Tettigidae (Subfamily of Aurigidæ)—continued							
Tettigidea parvipennis	13 spg 13 ♂ som 14 oog	7 ♂	6, 7 ♂		X to pole in 1st. Super-numerary X present in 1 an- imal in som and ♂ cells. To pole in 1st, same pole as X or the other	Robertson, '16 Robertson, '17	Jour. Morph., 27, p. 179 Kansas Univ. Sc. Bull. 10, p. 275
Tettigidea parvipennis pennata.....	13 spg 14 oog	7 ♂			X	Robertson, '16	Jour. Morph., 27, p. 179
Trimerotropis? fallax.....	23 spg	12 ♂	11, 12 ♂		X to pole in 1st	Carothers, '17	Jour. Morph., 28, p. 445
Trimerotropis? suffusa.....	24 ♀ som	12+1 super- numery				Wentich, '17	Jour. Morph., 28, p. 471
Trimerotropis suffusa.....							
d. Gryllidae (Part I, p. 58)							
Gryllotalpa borealis.....	23 spg 24 oog	12 ♂	11, 12 ♂	11, 12 ♂	XY to poles in 1st. X=2 elements	Payne, '16	Jour. Morph., 28, p. 287
Gryllotalpa vulgaris from Freiburg.....	12 spg	6 ♂			XY	Payne, '16	Jour. Morph., 28, p. 287
Gryllotalpa vulgaris from Naples.....	15 spg	8? ♂				Payne, '16	Jour. Morph., 28, p. 287
e. Locustidae (Part I, p. 59)							
Jamaicana flava.....	35 spg	18 ♂	17, 18 ♂	17, 18 ♂	X to pole in 1st 2 chroms may be fused	Woolsey, '15	Biol. Bull., 28, p. 163
Jamaicana subguttata.....	34, 35 spg	17, 18 ♂				Woolsey, '15	Biol. Bull., 28, p. 163
Jamaicana unicolor.....	33, 35 spg				2 pairs of chroms may be fused	Woolsey, '15	Biol. Bull., 28, p. 163 Tabulated in brief in Part I
Locusta viridissima.....	29 spg 29 ♂ som 30 oog 30 ♀ som	15 ♂	14, 15 ♂	14, 15 ♂	X to pole in 1st	Mohr, '16	Liège, 1916

III. COELENTERATA

a. Hydrozoa

1. Leptothecae

a. Anthomedusae (Part I, p. 83)

Clava leptostyla.....	12 ♀	12 ♀	12 ♀	G. T. Hargitt, '16	Jour. Morph., 27, p. 85
2. Trachylinae (Part I, p. 83)					
Aglaantha digitalis.....	16 oog	8 ♀	8 ♀	G. T. Hargitt, '17	Jour. Morph., 28, p. 593

PART II (1878-1918)

IV. ECHINODERMATA

a. Asterozoa

Asterias forbesii.....	177 ♀	177 ♀	177 ♀	Wilson and Mathews, '95	Jour. Morph., 10, p. 319
Asterias forbesii.....	187 pa cl	18 ♀	18 ♀	Tennent and Hogue, '06	J. Exp. Zool., 3, p. 517
Asterias forbesii.....	36 cl ca. 18 pa cl			May be A. vulgaris	Biol. Bull., 13, p. 309
Asterias forbesii.....	36 cl	18 ♀	18 ♀	Jordan, '07 Jordan, '08	Anat. Ans., 31, p. 39 Carnegie Inst. Pub. 102, p. 39
Asterias glacialis.....			8-9 ♂ pron	Field, '95	Jour. Morph., 11, p. 235
Asterias glacialis.....	18 cl 18 pa cl (auto-regulation)			Delage, '01	Arch. Zool. exp. et gen. ser. III, vol. 9, p. 285
Asterias glacialis.....	36 pa cl	18 ♀	18 ♀	Buchner, '11	Arch. Zellf., 6, p. 577
Asterias vulgaris.....	18 spg 18 cl 9 pa cl	9 ♂	9 ♂	2nd p. b. + egg = 36 May be A. forbesii	Biol. Bull., 13, p. 309
Cribrella sanguinolenta..	ca. 36 som			Jordan, '10	Arch. Zellf., 5, p. 394

b. Crinozoa

Antedon bifida.....	87 oog			Chubb, '06	Phil. Trans. Roy. Soc. London, 196 B, p. 447
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IV. ECHINODERMATA—Continued

SPECIES	DIPLOID AND PARtheno- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
c. ECHINOIDEA							
<i>Arbacia punctulata</i>	ca. 40 cl					Jordan, '12	J. Exp. Zool., 12, p. 391
<i>Arbacia pustulosa</i>	40 cl					Baltzer, '10	Arch. Zellf., 5, p. 497
<i>Echinus acutus</i>	38 cl					Doncaster and Gray, '11 Doncaster and Gray, '13	Proc. Camb. Phil. Soc. 16, p. 415 Q. J. M. S., 58, p. 483
<i>Echinus esculentus</i>	32 cl	16 ♀	16 ♀	16 ♀		Bryce, '03	Q. J. M. S., 46, p. 177
<i>Echinus esculentus</i>	38 cl					Doncaster and Gray, '11 Doncaster and Gray, '13	Proc. Camb. Phil. Soc. 16, p. 415 Q. J. M. S., 58, p. 483
<i>Echinus esculentus</i>	18 cl					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Echinus microtubercu- tus</i> var. <i>bivalens</i> (Bo- veri '06).....		18 ♀		18 ♀	In a few cases	Boveri, '00	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
<i>Echinus microtubercu- tus</i> var. <i>bivalens</i>	30+cl 16-18 ps cl	16-18 ♀			Pa eggs treated with strychnine	R. Hertwig, '88	Sitz. gesel. Morph. u. Physiol., München, 4, p. 99
						R. Hertwig, '95	Sitz. gesel. Morph. u. Physiol., München, 11, p. 41
						R. Hertwig, '96	Fest. Gegenbauer, 2, p. 21
<i>Echinus microtubercu- tus</i> var. <i>bivalens</i>	36 cl	18 ♀	18 ♀	18♂ in enu- cleated egg fragments		Stevens, '02	Arch. Entwickl., 15, p. 421
<i>Echinus microtubercu- tus</i> var. <i>bivalens</i>	36 cl 18 ps cl			18♂ in enu- cleated egg fragments 18 ♀	XY in ♂. From correction in '13	Baltzer, '09 Baltzer, '10 Baltzer, '13	Arch. Zellf., 2, p. 549 Arch. Zellf., 5, p. 497 Sitzungsber. Phys. med. Ges. Würzburg, 6, p. 90

<i>Echinus microtuberculatus</i> var. <i>univalens</i> (Boveri, '06).....	18 cl	♀♀	♀♀	9 ♀ 9 ♂ in enucleated egg fragments	Boveri, '90 Boveri, '05 Stevens, '02	Jan. Zeits., 17, p. 314 (Zellen-Studien III) Jan. Zeits., 31, p. 446 (Zellen-Studien V) Arch. Entwickl., 15, p. 421
<i>Echinus microtuberculatus</i> var. <i>univalens</i>	18 cl					
<i>Echinus microtuberculatus</i> var. <i>univalens</i> (= <i>Psammecchinus</i>).....	20+cl					
<i>Echinus microtuberculatus</i> var. <i>univalens</i>	18 cl			9-12 ♂ in enucleated egg fragments	Krabelska, '05	Bull. intern. de l'acad. des Sciences de Cracovie, '06, p. 49
<i>Echinus miliaris</i>	22 cl. 8-11 cl. in enucleated egg fragments fertilized			10-12 ♂ in enucleated egg fragment	Godlewski, '06 Morgan, '95	Arch. Entwickl., 20, p. 579 Arch. Entwickl., 2, p. 268
<i>Echinus miliaris</i>	30-34 cl					
<i>Echinus sphaera</i>	18 cl				Doncaster and Gray, '11 Doncaster and Gray, '13 Delage, '01 Jordan, '08	Proc. Camb. Phil. Soc., 16, p. 416 Q. J. M. S., 58, p. 483 Arch. Zool. exp. et gen., III, 9, p. 286 Carnegie Inst. Pub., 102, p. 39
<i>Hippoonoe seculenta</i>	327 cl			16-20 ♀	Pinney, '11 Tennant, '12 Pinney, '11 Retzius, '10	Biol. Bull., 21, p. 168 Jour. Morph., 23, p. 17 Biol. Bull., 21, p. 168 Biol. Untersuchungen, 15, p. 1
<i>Hippoonoe seculenta</i>	32-34 cl					
<i>Mouira atropos</i>	46 cl					
<i>Paracchinus miliaris</i>	18 pa cl					
<i>Psammecchinus</i> , see under <i>Echinus microtuberculatus</i>						
<i>Sphaerochinus granularis</i>		16-18 ♀			R. Hertwig, '96 Baltzer, '10	Fest. Gegenbaur, 2, p. 21 Arch. Zellf. 5, p. 497
<i>Sphaerochinus granularis</i>	40 cl (probably)					

IV. ECHINODERMATA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST CITE	2ND CITE	TID	REMARKS	OBSERVER	REFERENCE
<i>Sphaerochinus granularis</i>	18 ps cl					Godlewski, '12	Arch. Entwickl., 33, p. 196
<i>Strongylocentrotus lividus</i>	30+cl 16-18 ps cl	16-18 ♀			Pa eggs treated with strychnine	R. Hertwig, '98	Fest. Gegenbaur, 2, p. 21
<i>Strongylocentrotus lividus</i>	18 cl 18 ps cl (auto-regulation)			9 ♀		Delage, '99	Arch. Zool. exper. et gen. III, 7, p. 383
<i>Strongylocentrotus lividus</i>	36 cl			18♂ 18♀		Delage, '01	Arch. Zool. exper. et gen. III, 9, p. 286
<i>Strongylocentrotus lividus</i>						Boveri, '02	Verh. phys. med. Ges. sel. Würzburg, 36, p. 67
<i>Strongylocentrotus lividus</i>	36 cl					Petrunkewitsch, '04	Zool. Jahrb. suppl. 7, p. 77
<i>Strongylocentrotus lividus</i>	36 cl			18♂ 18♀	XY in ♂. From correction in '13	Baltzer, '09 Baltzer, '10 Baltzer, '13	Arch. Zellk., 2, p. 549 Arch. Zellk., 6, p. 497 Sitzungsber. Phys. Med. Gesel. Würzburg 6, p. 90
<i>Strongylocentrotus lividus</i>	36 cl					Schazel, '11	Arch. mikr. Anat., 76, p. 543
<i>Strongylocentrotus purpuratus</i>	36 cl 18 ps cl					Hindle, '11	Arch. Entwickl., 31, p. 146
<i>Toxopneustes variegatus</i>	14-24 cl					Selenka, '78	"Befruchtung des Eies von Fox. var. Leipsig.
<i>Toxopneustes variegatus</i>	36 cl 18 ps cl			18♂		Wilson, '85 Wilson, '01 Wilson, '01	Jour. Morph., 11, p. 443 Arch. Entwickl., 12, p. 329 Arch. Entwickl., 12, p. 363
<i>Toxopneustes variegatus</i>	36 cl				XY (in ♀?)	Hefner, '10	Biol. Bull., 19, p. 195
<i>Toxopneustes variegatus</i>	387 cl					Pinney, '11	Biol. Bull., 21, p. 168
<i>Toxopneustes variegatus</i>	36-38 cl 19 ps cl			18 or 19♂ in enucleated eggs	X or XY in ♂	Tennant, '12 Tennant, '13	J. Exp. Zool., 12, p. 391 J. Morph., 23, p. 17

d. HOLOTEUROIDEA

<i>Stichopus regalis</i> "and other Echinoderms"....	28-36 spg	16-18♂	8-9♂	Field, '93 Field, '95	Anat. Ans., 8, p. 487 Jour. Morph., 11, p. 226
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e. OPHUROIDEA

<i>Ophiocoma pumila</i>		ca. 18♀		Jordan, '08	Carnegie Inst. Pub. 102, p. 1
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V. MESOZOA

<i>Dioemenneca gracile</i>		ca. 30♀		Hartmann, '07	Mem. pub. par Cl. d. Sc. Acad. Roy. de Belgique, (47), 1, p. 1
<i>Haplozoon armatum</i>	100+som			Dogiel, '08	Zeit. wiss. Zool., 89, p. 417
<i>Rhopalura ophiocoma</i>	6 cl	3♀	3♀	Caulle et Laval- lée, '08	Arch. Zool. exp. et gen., Ser. IV, t. 8, p. 421

VI. MOLLUSCA

a. CEPHALOFODA

<i>Sepia officinalis</i>		6♀			Arch. d'Anat. microsc., 8, p. 239
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b. GASTROPODA

1. *Euthysanura*

a. Opisthobranchia

1. Nudibranchia

<i>Dialula sandiegensis</i>	32 cl	12♀		MacFarland, '97	Zool. Jahrb., 10, p. 227
<i>Doris bifida</i>		16♀	16♀	Smallwood, '05	Morph. Jahrb., 33, p. 87
<i>Montagus gouldii</i>	32 cl	16♀	16♀	Smallwood, '05	Morph. Jahrb., 33, p. 87
<i>Montagus pilata</i>		16♀	16♀	Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
<i>Phyllirhoe bucephala</i>	32 cl	16♀	16♀ 16♂ pron		
<i>Pleurophyllidia californica</i>	20-24 cl		10-12♀	MacFarland, '97	Zool. Jahrb., 10, p. 227

VI. MOLLUSCA—Continued

SPECIES	DIPLOID AND PARHENOGENETIC	1ST CYTE	2ND CYTE	-TID	REMARKS	OBSERVER	REFERENCE
2. Tectibranchia							
<i>Aplysia depilans</i>		16 ♀	16 ♀			Boehenek, '99	Bull. Acad. Sc. Cracovie 1899, p. 266
<i>Aplysia limacina</i>	24 cl (at least)					Carassi, '05	Arch. ital. anat. e emb. 4, p. 231
<i>Aplysia punctata</i>		16 ♀	16 ♀	16 ♀		Janssens and Ellington, '04	La Cellule, 21, p. 315
<i>Aplysia punctata</i>	24 cl (at least)					Carassi, '05	Arch. ital. anat. e emb. 4, p. 231
<i>Bulla solitaria</i> , see <i>Haminea</i>							
<i>Cressia acicula</i>	20 spg 20 oeg 20 som 20 cl 10 pa cl	10 ♂ 10 ♀	10 ♂ 10 ♀	9, 10 ♂ (those with ♀ not functional) 10 ♀	X to pole in 2nd	Zarnik, '11	Verh. d. deutsch. Zool. Gesell., 21, p. 205
<i>Cymbulia peronii</i>	32 cl	16 ♀				Nekrasof, '03 Nekrasof, '09	Anat. Anz., 24, p. 119 Arch. mikr. Anat., 73, p. 313
<i>Cymbulia peronii</i>	36 (not stated where)					Zarnik, '11	Verh. deutsch. Zool. Gesell., 21, p. 205
<i>Haminea solitaria</i> (= <i>Bulla solitaria</i>).....		16 ♀	16 ♀		Accessory chrom	Smallwood, '04	Bull. Mus. Comp. Zool. Harvard, 45, p. 269
<i>Hyalaea tridentata</i>	24 (not stated where)				X to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesell., 21, p. 205
<i>Hyalocylis striata</i>	24 (not stated where)	12 ♂	10-12 X (fused) ♂	10, 10 + 2 X (fused) ♂. Those with 10 degenerate	2X (fused) to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesell., 21, p. 205
<i>Tiedemannia neopolitana</i>	28 (not stated where)				X to pole in 2nd	Zarnik, '11	Verh. deutsch. Zool. Gesell., 21, p. 205

b. Pulmonata

Arion empiricorum.....	16-20 ♀				Platner, '86	Arch. mikr. Anat., 27, p. 32
Arion empiricorum.....	16-20 ♀				Garnault, '89	Zool. Anz., 12, p. 10
Arion empiricorum (or rufus).....	16 ♀				Lams, '10	Acad. roy. Belgique. Cl. d. Sc. Mem. Ser. 2, t. 2, no. 4, p. 1
Helix arbustorum.....	24♂	24♂	24♂		Soós, '10	Annales Mus. Nat. Hungarici, 8, p. 231
					Small heterochrom divides in 1st cell. Fate undetermined	
Helix arbustorum.....	24♂	24♂	24♂		Buresch, '11	Arch. Zellf., 7, p. 314
Helix aspersa.....	16-20 ♀				Garnault, '89	Zool. Anz., 12, p. 10
Helix hortensis (= Tschea h.).....	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445
Helix nemoralis.....	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445
Helix nemoralis.....	22♂, one animal had 23-29♂				Baltzer, '13	Arch. Zellf., 11, p. 161
Helix pomatia var. bivalens (Murray, '98)...	24♂	24♂	24♂		From correction in '11	
					Bolles-Lee, '98	La Cellule, 11, p. 223
					Bolles-Lee, '97	La Cellule, 12, p. 197
					Bolles-Lee, '99	La Cellule, 16, p. 47
					Bolles-Lee, '11	La Cellule, 27, p. 53
Helix pomatia var. bivalens.....	24♂	prob. 24♂	ca. 24♂		Murray, '98	Zool. Jahrb., 11, p. 427
Helix pomatia var. bivalens.....	24♂	24♂	2♂		Anceel, '02	Bibliogr. Anat., 11, p. 149
Helix pomatia var. bivalens.....	24♂	24♂	24♂		Anceel, '08	Arch. Biol., 19, p. 389
Helix pomatia var. bivalens.....	24♂	24♂	24♂		Tschasownikow, '05	Anat. Hefte, 29, p. 313
Helix pomatia var. bivalens.....	24♂	24♂	24♂		Kleinert, '09	Jen. Zeits., 38, p. 445

VI. MOLLUSCA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st CYTE	2ND CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Helix pomatia</i> var. <i>bi-valens</i>	ca. 48 spg	24♂	23, 24♂	23, 24♂. Those with 23 de- generate	One tetrad to pole in 1st	Demoll, '11 Demoll, '12 Demoll, '12	Zool. Anz., 38, p. 88 Zool. Jahrb. Suppl. 15, vol. 2, p. 107 Abt. Zool. Jahrb., Zool., 33, p. 40
<i>Helix pomatia</i> var. <i>uni-valens</i> (Murray, '98)....			24♂	12♂	Reduction in 2nd div.	Platner, '85 Platner, '89	Arch. mikr. Anat., 26, p. 599 Arch. mikr. Anat., 33, p. 134
<i>Helix pomatia</i> var. <i>uni-valens</i>	24 spg				May be sp. no.	Zimmerman, '91	Verh. Anat. Gesell., 5, p. 187
<i>Helix pomatia</i> var. <i>uni-valens</i>	24 spg	12♂ (=48 el)	12♂ (=24 el)	12♂	Tetrads and dyads consist of separate elements	Vom Rath, '92	Arch. mikr. Anat., 40, p. 102
<i>Helix pomatia</i> var. <i>uni-valens</i>	24 spg	12♂ (=48 el)	12♂ (=24 el)	12♂		Godlewski, '97	Bull. Acad. Sc. Cracovie, 1897, p. 68
<i>Helix pomatia</i> var. <i>uni-valens</i>	24 spg	12♂ (=48 el)	12♂ (=24 el)	12♂		Prowasek, '02	Arch. Zool. Inst. Wien, 13, p. 197
<i>Helix</i> (sp. not given).....	16? spg					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Limax agrestis</i>			16♂	8♂	Reduction in 2nd div.	Platner, '89	Arch. mikr. Anat., 33, p. 126
<i>Limax cinereo-niger</i>	16 spg	8♂ (=32 el)	8♂ (=16 el)	8♂	Tetrads and dyads consist of separate elements	Vom Rath, '92	Arch. mikr. Anat., 40, p. 102
<i>Limax maximus</i>		ca. 20 ♀				Washburn, '94	Amer. Nat., 28, p. 528
<i>Limax maximus</i>		16-20 ♀ (prob. 16)	16 ♀			Linville, '00	Bull. Mus. Comp. Zool. Harvard, 35, p. 211
<i>Limnea elodae</i>		16 ♀	16 ♀			Linville, '00	Bull. Mus. Comp. Zool. Harvard, 35, p. 211
<i>Tachea austriaca</i> (= <i>Helix</i> a.).....		25♂				Baltzer, '13	Arch. Zellf., 11, p. 151
<i>Tachea hortensis</i> (= <i>Helix</i> b.).....	44 spg 40-48 pa (?) spg	22♂, (Per- haps 22 ♀) 22 pa (?)			Questions if pa. or self-fertilised	Baltzer, '13	Arch. Zellf., 11, p. 151

1. *Streptoneura* (= *Probranchia*)
a. *Pectinobranthia*
1. Heteropoda

<i>Carinaria mediterranea</i>	32 cl	16 ♀	16 ♀ 16 ♂ pron	Boveri, '90	Jen. Zeits., 17, p. 314 (= Zellen-Studien III)
<i>Columbella rustica</i>		16+♂		Schütz, '17	Arch. Zool. exper. et gen. Notes et Rev., 56, p. 32
<i>Enteroneus oestergreni</i>	34 oog	17 ♀	17 ♀ 17 ♂	Bonnevie, '05	Anat. Ans., 26, pp. 374 and 407
<i>Enteroneus oestergreni</i>	42 som	21 ♂ 21 ♀	21 ♂ pron 21 ♀ pron	Bonnevie, '06 Schreiner, '07	Jen. Zeits., 34, p. 229 Videnak-Selak. Skr. Math.-Naturv., 1907, no. 2, p. 1
<i>Pterotrachea mutica</i>	32 cl	16 ♀	16 ♀ 16 ♂ pron	Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)

2. Platyzoa

<i>Bythina tentaculata</i>	22-28 spg	24-28 ♂		Fusion of chroms in oligopyrene	Arch. Zellf., 12, p. 567
<i>Conus mediterranea</i>		14 ♂		Von Kemnitz, '14	Kuschakewitsch, '13
<i>Crepidula plana</i>	60 cl	30 ♀		Conklin, '02	Jour. Acad. Nat. Sc. Phila., 12, p. 1
<i>Fulgur carica</i>		Prob. 16 ♀		McMurrich, '06	Anat. Ans., 12, p. 534
<i>Paludina vivipara</i>	16 fuse to form 4 spg	4 ♂ (= 16 cl)	4 ♂ (= 8 cl)	Auerbach, '06	Jen. Zeits., 23, p. 405
<i>Paludina vivipara</i>	14 spg	7 ♂	7 ♂	Méves, '01	Verh. Anat. Gesell., 1901, p. 23
<i>Paludina vivipara</i>	14 oog 14 som 14 cl	7 ♀		Méves, '03	Arch. mikr. Anat., 61, p. 1
<i>Valvata piscalis</i>	ca. 20 spg	10 ♂		Popoff, '07	Arch. mikr. Anat. 70, p. 43
<i>Vermetus gigas</i>		14 ♂		Popoff, '08	Biol. Centralb., 28, p. 555
				Von Kemnitz, '14	Arch. Zellf., 12, p. 567
				Kuschakewitsch, '13	Arch. Zellf., 10, p. 237

VI. MOLLUSCA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>c. LAMELLIBRANCHIA</i>							
<i>Cumingea tellinoides</i>		18 ♀	18 ♀			Jordan, '10	Arch. Zellf., 4, p. 243
<i>Cumingea tellinoides</i>	Prob. 36 cl 18 ps cl (2 p. b.'s) 80-60 ps. cl. (no p. b.'s)	18 ♀	18 ♀			Morris, '17 Morris, '18	J. Exp. Zool., 22, p. 1 Biol. Bull., 35, p. 260
<i>Macra</i>	24 cl 12 ps cl	12 ♀	12 ♀	12 ♀	One or both p. b.'s may be retained in ps. causing va- riations in chrom no.	Kostanecki, '04 Kostanecki, '04 Kostanecki, '11	Arch. mikr. Anat., 64, p. 1 Bull. Acad. Sc. Craco- vie, 1904, p. 70 Arch. mikr. Anat., 78, Abt. II, p. 1
<i>Unio</i>		16 ♀	16 ♀			Lillie, '01	Jour. Morph., 17, p. 227

VII. MOLLUSCOIDEA

a. BRACHIOFODA

<i>Lingula anatina</i>				8♂ upon 8♀		Yatai, '02	Jour. Coll. Sc. Imp. Univ. Tokyo, 17, art. 4, p. 1
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b. BRYOZOA

1. *Ectoprocta*

<i>Membranipora pilosa</i>		11 ♀				Bonnevie, '06 Bonnevie, '07	Arch. f. Mathem. og Naturv., 27, no 13 Jen. Zeits., 35, p. 567
<i>Plumatella fungosa</i>	5 cl	6 or 7♂				Braem, '97	Zoologica, 10, Hefte 23, p. 1

3. *Endoprocta*

<i>Pedocellina americana</i>	22 spg 22 oog 22 cl	11♂ 11♀	11♂ 11♀	Dublin, '05	Annals N. Y. Acad. Sci., 16, p. 1
<i>Pedocellina echinata</i>		8♀		Lebedinsky, '05	Biol. Centralb., 25, p. 336

c. PHORONIDA

<i>Phoronis australis</i>		12♂ 13♀		Ikeda, '03	Annot. Zool. Japan, 4, p. 141
<i>Phoronis iijimai</i>		6♂ 6♀	3♂ 3♀	Ikeda, '01	Jour. Coll. Sc. Imp. Univ. Tokyo, 13, p. 597
				Ikeda, '03	Annot. Zool. Japan, 4, p. 141

VIII. NEMATHELMINTHES

a. ACANTHOCEPHALA

<i>Echinorhynchus acus</i>	8 som	8♀		Hamann, '91	Jen. Zeits., 18, p. 113
<i>Echinorhynchus gigas</i>		4♂		Kaiser, '93	Bibliot. Zool., 7, Part II, p. 1
<i>Echinorhynchus haerues</i> } <i>Echinorhynchus poly-</i> } <i>morphus</i>	8 cl	8♀		Hamann, '91	Jen. Zeits., 18, p. 113
<i>Echinorhynchus proteus</i>	6 spg	4♀		Von Voss, '10	Arch. Zellf., 5, p. 430
<i>Gigantorhynchus gigas</i>		3♂		Noé, '10	Arch. ital. de Biol., 53, p. 315
<i>Gigantorhynchus hirudi-</i> <i>nareus</i>	6 spg	3♂	3♂	Noé, '14	Mem. d. R. Acad. Lincei Ser. 5, vol. 10, p. 46

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CTE	2ND -CTE	-TID	REMARKS	OBSERVER	REFERENCE
b. CHAETOGNATHA							
<i>Sagitta bipunctata</i>		8♂	8♂	4♂		Bolles-Lee, '88	La Cellule, 4, p. 105
<i>Sagitta bipunctata</i>	18 cl	9♀	9♀	9♀ pron		Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
<i>Sagitta bipunctata</i>	18 spg 18 som	9♂ 9♀	9♂ 9♀	9♂ 9♀		Stevens, '03	Zool. Jahrb., 18, p. 227
<i>Sagitta bipunctata</i>	18 cl	9♀	9♀	9♀		Stevens, '10	Jour. Morph., 21, p. 279
						Buchner, '09	Anat. Ans., 35, p. 433
						Buchner, '10	Fest. R. Hertwig 1, p. 235
<i>Sagitta bipunctata</i>	18 som	9♀				Elpatiewsky, '10	Biol. Zeits. Moskou, 1, p. 333
<i>Sagitta bipunctata</i>	18 spg	9♂	9♂			Bordas, '12	La Cellule, 28, p. 165
						Bordas, '14	Mém. d. l. Réal. Soc. d'Hist. nat. d'Esp., d. Hist. nat. 10, p. 1
<i>Sagitta elegans</i>	18 som	9♂ 9♀	9♂	9♂		Stevens, '05	Zool. Jahrb., 21, p. 243
						Stevens, '10	Jour. Morph., 21, p. 279
<i>Sagitta inflata</i>		9♂	9♂	9♂		Stevens, '10	Jour. Morph., 21, p. 279
<i>Sagitta inflata</i>	18 cl	9♀	9♀	9♀		Buchner, '10	Fest. R. Hertwig 1, p. 235
<i>Sagitta minima</i>		9♂	9♂	9♂		Stevens, '10	Jour. Morph., 21, p. 279
c. NEMATODA							
1. Gordiidae							
<i>Gordius affinis</i>	4 oog	1♀			4 chroma form 1 tetrad	Švábenik, '09	Sitzb. Kon. Böhm. Gesell. d. Wissensch. Prag, 1909, art. 7
<i>Gordius aquaticus</i>	7-9 cl					N. Th. Meyer, '13	Zeit. wiss. Zool., 105, p. 128
<i>Gordius aquaticus</i>	4 cl					Muhldorf, '13 Muhldorf, '14	Zool. Ans., 42, p. 431 Zeit. wiss. Zool., 111, p. 1

<i>Gordius gratianopolensis</i>		8♀				4 chroms form 1 tetrad	Camerano, '90	Mem. R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
<i>Gordius montenigrinus</i>	4 oog	1♀			1♂		Svábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
<i>Gordius preslii</i>	4 spg						Vejdovsky, '94 Vejdovsky, '12	Zeit. wiss. Zool., 87, p. 643 Kon. Böhm. Gesel. Wissen. Prague, p. 1
<i>Gordius preslii</i>	4 oog	1♀				4 chroms form 1 tetrad	Švábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
<i>Gordius toboeanus</i>		8♀					Camerano, '90	Mem. R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
<i>Gordius toboeanus</i>	4 oog	1♀				4 chroms form 1 tetrad	Švábenik, '09	Sitz. Kon. Böhm. Gesel. d. Wissen. Prague, 1909, art. 7
<i>Gordius toboeanus</i>	4 spg 4 oog	2♀					Vejdovsky, '12	Kon. Böhm. Gesel. Wissen. Prague, p. 1
<i>Gordius villosi</i>		8♀					Camerano, '90	Mem. R. Accad. Sc. Torino, Ser. II, vol. 40, p. 1
<i>Paragordius varius</i>	14 cl	7♀	7♀	7♀	7♀ pron. 7♂ pron.		Montgomery, '04	Proc. Acad. Nat. Sc. Phila., 56, p. 733

2. *Nematoidea*

<i>Ancyracanthus cystidicola</i>	11 spg 12 oeg 11♂ cl 12♀ cl 11♂ som	6♂ 6♀	5, 6♂ 6♀	5, 6♂ and ♂ 6♀ and ♀ pron.	X to pole in 1st	Mulsow, '11 Mulsow, '12	Zool. Anz., 28, p. 484 Arch. Zellf., 9, p. 63
<i>Angiostomum nigrovirens</i> (= <i>Acaris nigrovirens</i>)		6♀ (sometimes 5)	6♀ (sometimes 5)		Hermaphroditic generation	McDowall, '06 McDowall, '08	Proc. Camb. Phil. Soc. 13, p. 209 Proc. Camb. Phil. Soc. 14, p. 613

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st-CYTE	2ND-CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Angiostomum nigrov- osum</i> (= <i>Rhabdonema nigrovosum</i>)	12 oog	7♂, sometimes 6, hetero- chroma united 6♀	7♂ 6♀	6♂. Later 5, 6, hetero- chrom dis- carded in half the cells. One case 5, 7, both hetero- chroms to same pole 6♀	Hermaphroditic generation. heterochroms to opposite poles in 2nd ♂		
<i>Ascaris canis</i> (<i>A. mystax</i>).	11 emb. germ cells (♂?) 22 emb. som cells (♂?) 12 emb. germ cells (♀?) 24 emb. som cells (♀?) 22 spg 22 oeg 22 el 11 (tetrad) prim. germ cells	11♂ 11♀	11♂ 11♀	11♂ 11♀	Chrom diminution in som cells. Walton '16 says this is <i>A. triquetra</i>	Marcus, '05 Marcus, '06	Sitz. Ber. Gesel. Morph. u. Physiol. München 21, p. 39 Arch. mikr. Anat., 68, p. 441
<i>Ascaris canis</i>	30 spg 26 oeg 30, 36 som	18♂ 18♀	12, 18♂ 18♀	12, 18♂ 18♀	X (=6 el) to pole in 1st. Fragmenta- tion in som cells into 60 (in ♂) or 72 (in ♀) monad chroms	Walton, '16 Walton, '16 Walton, '18	Jour. Parasitol., 3, p. 39 Biol. Bull., 31, p. 364 Jour. Morph., 30, p. 527
<i>Ascaris clavata</i>		24♀	24♀	24♀	In 2 cases, no. is double	Carnoy, '86	La Cellule, 3, p. 229
<i>Ascaris</i> of dog (not <i>A. mystax</i>)		2 groups of 4♀	1 group of 4♀	4 (divided) ♂ pron		Carnoy, '86	La Cellule, 3, p. 1

<i>Ascaris des Hundes</i>	16 ♀	8 ♀	4 ♀	Uncertain if same as Carnoy's	Lukjanow, '89	Arch. mikr. Anat., 34, p. 387
<i>Ascaris felis</i>	9♂	9♂	9♂	XY to poles in 1st	Edwards, '12	Arch. Zellk., 7, p. 309
<i>Ascaris felis</i>	9♂	9♂	9♂	XY (or X attached to autosome) to poles in 1st	Walton, '16	Biol. Bull., 31, p. 364
<i>Ascaris inourva</i>	21♂ 21 ♀	14, 21♂ 21 ♀		XY to poles in 1st. X=8 el. Chromatin diminution in somatic cl.	Goodrich, '14 Goodrich, '16	Biol. Bull., 27, p. 147 Jour. Exp. Zool., 21 p. 61
<i>Ascaris lumbricoidea</i>	20-24 ♀ (prob. 24)	24 ♀	24 ♀		Carnoy, '86	La Cellule, 3, pp. 1, 229
<i>Ascaris lumbricoidea</i>	24 ♀ (sometimes 25)	24 ♀	24 ♀		Boveri, '86	Sitz. Ber. Gesell. Morph. u. Physiol. München, 2, p. 101
<i>Ascaris lumbricoidea</i>	48-50 cl		24♂ pron 24 ♀ pron	Chromatin diminution and breaking of chroma in somatic cleavages	Boveri, '87	Jen. Zeits., 14, p. 423 (Zellen-Studien I)
<i>Ascaris lumbricoidea</i>					Bonnevie, '02	Jen. Zeits., 29, p. 275
<i>Ascaris lumbricoidea</i>	24♂ 24 ♀	19, 24♂ 24 ♀	19, 24♂	X to pole in 1st. X=6 el	Edwards, '10 Edwards, '10	Science, 31, p. 514 Arch. Zellk., 6, p. 422
<i>Ascaris megalocephala</i> var. <i>bivalens</i> (Hertwig, '90).....	2 ♀		2 ♀		Schneider, '83	'Das Ei und Seine Befruchtung.' Breslau
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4♂ 4 ♀	4 ♀		Diminution and fragmentation of chroms in som cl	Nussbaum, '84 Nussbaum, '02	Arch. mikr. Anat., 23, p. 185 Arch. mikr. Anat., 59, p. 647
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	2♂ 2 ♀	2♂ 2 ♀	2♂ pron 2 ♀ pron.		Van Beneden, '83 (84) Van Beneden and Julin, '84	Arch. Biol., 4, p. 265
					Van Beneden and Neyt, '87	Bull. Acad. Roy. des Sc. de Belgique, Ser. III, t. 7, p. 312
					Van Beneden, '88	Bull. Acad. Roy. des Sc. de Belgique, Ser. III, t. 14, p. 215
					Van Beneden, '88	Anat. Ans., 3, p. 104

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARATHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 cl	2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ pron 2 ♀ pron		Carnoy, '86 Carnoy, '86 Carnoy and Le- brun, '97	La Cellule, 2, p. 1 La Cellule, 3, pp. 1 and 229 La Cellule, 13, p. 61
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 cl	2 ♀	2 ♀	2♂ pron 2 ♀ pron 3♂ pron (3 cases)	Diminution and fragmentation of chroms in som cl X sometimes fused with other chrom., sometimes sepa- rate	Boveri, '87 Boveri, '87 Boveri, '87 Boveri, '88 Boveri, '04 Boveri, '09 Boveri, '09 Boveri, '10	Sitz. Gesell. Morph. u. Physiol. Munchen, 3, p. 71 Anat. Ans., 2, p. 688 Jen. Zeits., 14, p. 493 (=Zellen-Studien I) Jen. Zeits., 13, p. 685 (=Zellen-Studien II) "Ergeb. Konstitution d. chrom. Substanz d. Zellkerns." Jena Arch. Zell., 3, p. 131 Arch. Zell., 4, p. 135 Fest. R. Hertwig, III, p. 129
<i>Ascaris megalocephala</i> var. <i>bivalens</i>		2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ pron 2 ♀ pron		Van Gehuchten, '87	Anat. Ans., 2, p. 761
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 cl	2 ♀ (=8 el)	2 ♀ (=4 el)	2♂ pron 2 ♀ pron 2 ♀ pron	Also 'parachromo- somes'	Zacharias, '87 Zacharias, '87 Zacharias, '12 Zacharias, '12	Anat. Ans., 2, p. 787 Arch. mikr. Anat., 30, p. 111 Anat. Ans., 42, p. 363 Zool. Ans., 40, p. 25
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 cl			2♂ pron 2 ♀ pron		Dostoiewsky, '88	Anat. Ans., 3, p. 646
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 cl	2 groups of 4 each ♀	2 groups of 2 each ♀	2♂ 2 ♀ 2♂ pron		Kulschitzky, '88 Kulschitzky, '88	Sitz. k. Akad. wissen. Berlin, 88, p. 17 Arch. mikr. Anat., 31, p. 567
<i>Ascaris megalocephala</i> var. <i>bivalens</i>	4 spg 4 oog	2♂ 2 ♀	2♂	2♂		Hertwig, '90	Arch. mikr. Anat., 36, p. 1

<i>Ascaris megalocephala</i> var. bivalens.....	4 spg	2♂	2♂	2♂	Brauer, '93 Arch. mikr. Anat., 42, p. 153
<i>Ascaris megalocephala</i> var. bivalens.....	4 sex cells				Von Wasiclewski, '93 Arch. mikr. Anat., 41, p. 324
<i>Ascaris megalocephala</i> var. bivalens.....	4 (or 2 double) cl. Sometimes 6. Sometimes fragmentation inprim. germ cells, giving 8- 12+				Vom Rath, '94 Biol. Centralb., 14, p 449
<i>Ascaris megalocephala</i> var. bivalens.....	4 cl. Some- times frag- mentation or double fert., giving 5-6				Herla, '95 Arch. Biol., 13, p. 423
<i>Ascaris megalocephala</i> var. bivalens.....	4 cl 2 cl (anomaly)				Zoja, '96 Arch. mikr. Anat., 47, p. 218
<i>Ascaris megalocephala</i> var. bivalens.....	4 oog	2♀	2♀	2♀	Sabashnikoff, '97 Bull. Soc. Imp. d. Nat. d. Moscou, 9, p. 82
<i>Ascaris megalocephala</i> var. bivalens.....		2♀	2♀	2♀	Mooskowi, '02 Arch. mikr. Anat., 59, p. 388
<i>Ascaris megalocephala</i> var. bivalens.....	4 cl	2♀	2♀	2♀ pron 2♂ pron	Montgomery, '04 Biol. Bull., 6, p. 137 Montgomery, '08 Arch. Zellf., 2, p. 66
<i>Ascaris megalocephala</i> var. bivalens.....	4 cl	2♂ 2♀	2♀	2♀	Tretjakoff, '05 Arch. mikr. Anat., 65, pp. 388 and 393
<i>Ascaris megalocephala</i> var. bivalens.....		2♀	2♀	2♀	Griggs, '06 Ohio Naturalist, 6, p 519
<i>Ascaris megalocephala</i> var. bivalens.....	5 spg 5 cl				Boring, '09 Arch. Zellf., 4, p. 120
					One small chrom in some eggs of most worms, due to fragmentation or a sex chrom

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Ascaris megalocephala</i> , var. bivalens.....	5 spg	3♂	3♂ or 2.3	2, 3♂	X to pole in 1st or 2nd (3 worms)	Edwards, '10	Science, 31, p. 514
<i>Ascaris megalocephala</i> , var. bivalens.....	4 cl	2♀	2♀	2♀ 2♂ pron		Edwards, '10	Arch. Zellf., 5, p. 422
<i>Ascaris megalocephala</i> , var. bivalens.....	4 cl			2♂ pron		Retsius, '11	Biol. Untersuchungen, 16, p. 21
<i>Ascaris megalocephala</i> , var. bivalens.....	1-8 cl			2♂ pron		Vejdovsky, '12	König. Böhm. Gesel. Wissen. Prag, p. 1
<i>Ascaris megalocephala</i> , var. bivalens.....	27♂ (?) emb. 36♀ (?) emb.			1-2♂ pron	Abnormalities in cl. (one animal).	Kautzsch, '12	Arch. Entwickl., 35, p. 642
<i>Ascaris megalocephala</i> , var. bivalens.....	5 cl (♂?) 6 cl (♀?) or 4 cl	3♀ or 2	3♀ or 2	3♀ or 2 2 or 3♂ pron	X separate or fused with another chrom	Frolowa, '12	Arch. Zellf., 9, p. 149
<i>Ascaris megalocephala</i> , var. bivalens.....	4 spg 4 oog 4 cl	2♂ 2♀	2♂ 2♀	2♂ 2♀		Fauré Fremiet, '13	Arch. d'Anat. mikr., 15, p. 435
<i>Ascaris megalocephala</i> , var. bivalens.....	4 oog		2♀			Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Ascaris megalocephala</i> , var. bivalens.....		2♀				de Saedeleer, '13	La Cellule, 28, p. 301
<i>Ascaris megalocephala</i> , var. bivalens.....	2-7 cl	4♀, rarely 5 due to piece from 1 chrom. bro- ken off	4♀, some- times 8, no 1st p. b.; 5 or 6 due to frag- ments	2♀, also sev- eral due to fragmenta- tion	One animal. Dyads instead of tetrads	Geinitz, '15	Arch. Zellf., 13, p. 588
	4 cl (no X) 5 cl (1 X) 6 cl (2 X) 7 cl (X and only 1 p. b.) ca. 52♂ emb (49-54) ca. 60♀ emb (58-65)	3♀	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	Three animals. X (double) to pole in 1st or 2nd, or di- vides in both. X -8 el in embryos. Rarely X-4 el		

<i>Ascaris megalocephala</i> , var. <i>trivalens</i> (Zacharias, '12).....	3 cl			1♂ (?) pron 2 ♀ (?) pron	Probably cross biv. X univ. Sometimes small ac- ces. chrom	Zacharias, '12	Biol. Centrall., 32, p. 718
<i>Ascaris megalocephala</i> , var. <i>univalens</i> (Hertwig, '90).....	2 cl	1 ♀	1 ♀	1 ♀ 1♂ pron	Chromatin diminution and fragmen- tation of chroms in som cells	Roveri, '87 Roveri, '88 Roveri, '92 Roveri, '99 Roveri, '04 Roveri, '09	Jen. Zeits., 14, p. 423 (Zellen-Studien I) Jen. Zeits., 15, p. 685 (Zellen-Studien II) Sitz. Gesel. Morph. u. Physiol. München 8, p. 114 Fest. Von Kupffer, p. 383 "Ergeb. Konstitution d. chrom. Substanz d. Zellkerns," Jena Arch. Zellf., 3, p. 181
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl			1♂ pron 1 ♀ pron		Dostolewsky, '88	Anat. Ans., 3, p. 646
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl					Kultschitzky, '88	Arch. mikr. Anat., 31, p. 867
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg 2 oog	1♂	1♂	1♂		Hertwig, '90	Arch. mikr. Anat., 36 p. 1
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg 2 oog 2 cl	1♂ 1 ♀	1♂ 1 ♀	1♂ 1 ♀		Schneider, '91	Arch. mikr. Anat., 36 p. 179
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 sex cells					Von Wastielewski, '93	Arch. mikr. Anat., 41, p. 324
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg	1♂	1♂	1♂		Brauer, '93	Arch. mikr. Anat., 42, p. 163
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl	4 el ♀	2 el ♀	1 ♀ 1♂ pron		Herla, '95	Arch. Biol. 13, p. 423
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 .l			1♂ pron 1 ♀ pron		Carney and Le- brun, '97	La Cellule, 13, p. 61
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 sex cells 2 cl				Diminution and fragmentation of chroms in som ol	Nussbaum, '02	Arch. mikr. Anat., 59, p. 647

<i>Filaria papillosa</i>	11♂ cl 12♀ cl	6♀	6♀	5, 6♂ 2♀ Prob. 4♂ pron		Meyer, '15	Arch. mikr. Anat., 87, II, p. 12
<i>Filaroides mustelorum</i> ...	16 cl	8♀	4♀			Carnoy, '86	La Cellule, 3, pp. 1 and 63
<i>Heterakis</i> sp. 2.....	9 spx	4♂ 5♀	4, 5♂ 5♀		X to pole in 1st	Boveri, '09	Arch. Zellf., 4, p. 136
<i>Heterakis dispar</i>	9 spx 10 oog	4♂ 5♀	4, 5♂ 5♀		X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Heterakis inflata</i>	10 spx	4♂ 5♀	4, 5♂ 5♀		X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Heterakis vesicularis</i>	9 spx 10 oog	5♂ 5♀	4, 5♂ 5♀		X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Ophioctenostomum mucronatum</i>	12 cl	6 (double) ♀	6 (double) ♀	6♀ 4+♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
<i>Oxyuris ambigua</i>	2-47 spx	1-3♀	1-3♀	1-3♀		Löwenthal, '89 Löwenthal, '90	Inter. Monats. Anat. u. Physiol., 6, p. 364 Inter. Monats. Anat. u. Physiol., 7, p. 375
<i>Rhabditis</i> sp. 1.....	18 cl 18 som	10♂ (= 8 biv. + 2 univ.) Rarely 9 biv. no univ. 18♀	10♂	9♂ 18♀	Only 1 p. b. Sperm degenerate in eggs, really pa. XY to poles in 2nd generation. Occasionally one sex chrom dis- carded in Rest- körper	Kruger, '12 Kruger, '13	Zool. Anz., 40, p. 223 Zeit. wiss. Zool., 105, p. 87
<i>Rhabditis</i> sp. 2.....	11 cl (♂?) 12 cl (♀?)	6♂ 6♀	5, 6♂ 6♀	5, 6♂ (sperm with 5 not functional) 6♀	Separate generations X to pole in 1st	Boveri, '11	Verh. phys. med. Ge- sell. Würzburg, 41, p. 83
<i>Rhabditis</i> sp. 3.....	13 cl 13 ooc 13 abd 12 oog 12 som ♀	5+2 X♂ 6♀	5+2 X♂ 6♀	5, 6, 7♂ (sperm with 7 degenerate) 6♀	Hermaphroditic generation 2 X to pole in 2nd or 3rd X to each pole		
<i>Rhabditis</i> sp. 4.....	13 cl	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀			
<i>Rhabditis</i> sp. 5.....	11 spx 12 oog	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀	X to pole in 1st or 2nd	Kohls, '13	Arch. mikr. Anat., 83, Abt. II, p. 191
<i>Sclerosomum</i> (Scleron- gylus) edentatum.....	11 spx 12 oog	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀			
<i>Sclerosomum equinum</i>							
<i>Sclerosomum vulgare</i>							

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARHENO- GENETIC	1ST -OTYB	2ND -OTYB	-TID	REMARKS	OBSERVER	REFERENCE
<i>Spioptera strumosa</i>	12 cl	8 ♀	4 ♀	2 ♀ 4 ♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
<i>Strongylus</i> , see <i>Solero- stomum</i>							
<i>Strongylus flaria</i>	12 spg 12 oog 12 cl	6 ♂ 6 ♀	6 ♂	6 ♂ 6 ♀		Struckmann, '06	Zool. Jahrb., 22, p. 577
<i>Strongylus paradoxus</i>		6 ♀				Struckmann, '06	Zool. Jahrb., 22, p. 577
<i>Strongylus paradoxus</i>	11 spg 12 oog 11 cl ♂ 12 cl ♀	6 ♂ 6 ♀	5, 6 ♂ 6 ♀	5, 6 ♂ 6 ♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Strongylus tenuis</i>		6 ♂	6 ♂	5, 6 ♂	X to pole in 2nd	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Strongylus tetraacanthus</i> ..		6 ♀				Meyer, '05	Jen. Zeits., 22, p. 391

IX. NEMERTINEA
a. DITYARIA

<i>Tetrastemma vermiculus</i> .		4 ♀	2 ♀	2 ♀		Lebedinsky, '97 Lebedinsky, '97	Arch. mikr. Anat., 49, p. 503 Biol. Centralb., 17, p. 113
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b. TRITYARIA

<i>Cerebratulus lacteus</i>			5 ♀ (in p. b.)			C. B. Wilson, '00	Q. J. M. S., 43, p. 97
<i>Cerebratulus lacteus</i>	36 or 38 cl	18 or 19 ♀				Yatsu, '07 Yatsu, '09	Biol. Bull., 13, p. 300 Jour. Morph., 20, p. 353
<i>Cerebratulus marginatus</i> .	32 cl	16 ♀	16 ♀	16 ♀		Coe, '99	Zool. Jahrb., 12, p. 425
<i>Cerebratulus marginatus</i> .		16 ♂	16 ♀	16 ♀		Kostanecki '02	Bull. Inter. Acad. Sc. Cracovie 1902, p. 270

<i>Lineus geseerensis</i>	22 ♀ cl	8 ♀	8 ♀	Arnold '99	Trav. Soc. Imp. Nat. St. Petersburg no. 9, p. 1
<i>Lineus lacteus</i>		16♂ ♀		Meek '13	Phil. Trans. Roy. Soc. London 203B, p. 1.
<i>Lineus ruber</i>	16 cl	8 ♀ (=22 cl)	8 ♀ pron	Nusbaum and Orner, '13	Zeit. wiss. Zool., 107, p. 78
<i>Micurus caesus</i>	22 cl	16 ♀		Coe, '99	Zool. Jahrb., 12, p. 425

X. PLATHELMINTHES

a. Cestoda

<i>Avitellina centripunctata</i>	12-14 ? som	4? ♀		Gough, '11	Q. J. M. S., 86, p. 317
<i>Moniezia expansa</i>	}	6-9 ? ♂		Child, '07	Biol. Bull., 12, pp. 89, 191
<i>Moniezia planilima</i> ... }				Von Janicki, '07	Zeit. wiss. Zool., 87, p. 686
<i>Taenia serrata</i>		6-16 ♀ (prob. 12-16 ♀)			

b. Trematoda

1. Digenea

<i>Bilharzia haematobia</i> , see <i>Schistosomum haematobium</i>	20 spg 20 oog 20 cl	16♂ 10 ♀		Von Kennitz, '13	Arch. Zellf., 10, p. 470
<i>Brachycoelium salmandrae</i> (= <i>B. crassicoele</i>)...					
<i>Dicrocoelium lanceatum</i> (= <i>Distomum lanceolum</i>).....	20 oog	10 ♀	10 ♀	Goldschmidt, '08	Arch. Zellf., 1, p. 232
<i>Dicrocoelium lanceatum</i> (= <i>Distomum lanceolum</i>).....	20 spg	10♂	10♂	Dingler, '10	Arch. Zellf., 4, p. 672
<i>Diplostomum temporatus</i> ...	16 ps cl	16 ♀	One p. b. for ps.	Cary, '06	Zool. Jahrb., 28, p. 595
<i>Distomum hepaticum</i> ...		8-8 ♀		Hennequy, '06	Arch. d'Anat. micros., 9, p. 47
<i>Distomum hepaticum</i> , see <i>Fasciola hepatica</i>					

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1st CYTE (♀-♀)	2ND CYTE	7TD	REMARKS	OBSERVER	REFERENCE
<i>Platynum laticolatum</i> sp. <i>Diococelum lan-</i>	18 cl	9 ♀	9 ♀	9 ♀		Levy, '14	Arch. mikr. Anat., 85, Abt. II, p. 125
<i>Platynum turgidum</i>	8 cl			4♂ pron 4 ♀ pron		Schubmann, '05	Zool. Jahrb., 21, p. 571
<i>Fasciola hepatica</i> (a <i>Di-</i> <i>stomum hepaticum</i>)	12 oeg 12 cl	6 ♀, some each 12 ♀, no reduc- tion	6 ♀	6 ♀		Schellenberg, '11	Arch. Zellf., 6, p. 443
<i>Fasciola hepatica</i>	10 (pairs) spg 10 (pairs) oeg	10 (pairs) ♂ 10 (pairs) ♀	5 (pairs) ♂	5 (pairs) ♂	5 pairs to each pole in 1st div.	Dehorne, '11	Arch. Zool. exp. et gen. Ser. V, t. 9, p. 1
<i>Schistosomum haematobium</i> (= <i>Bilharzia haematobia</i>)	14 spg	8♂ 8 ♀	6, 8♂	6, 8♂	2 X to pole in 1st	Lindner, '14	Arch. Zellf., 12, p. 516
<i>Zoogonous mirus</i>	10 spg 10 oeg 10 cl	10 ♀	10 ♀	5 ♀ 5♂ pron	Reduction in 2nd div. 5 to each pole	Goldschmidt, '05 Goldschmidt, '08	Zool. Jahrb., 21, p. 607 Arch. Zellf., 2, p. 343
<i>Zoogonous mirus</i>	20+cl 22-26 som	11-13 ♀	11-13 ♀	11-13 ♀ 10-13♂ pron		Schreiner, '08	Skr. Vidensk-Selsk. Christiania, Math. Naturv., 1, no. 5, p. 1
<i>Zoogonous mirus</i>	12 spg 12-14 cl (prob. 12 cl)	6♂ 6 ♀	6 ♀	6♂ pron 6 ♀		Gregoire, '09	La Cellule, 25, p. 243
<i>Zoogonous mirus</i>	11-14 oeg (prob. 12 oeg)	6-7 ♀	6 ♀	7♂		Wassermann, '11	Sitz. Gesell. Morph. u. Physiol. München, 27, p. 128
<i>Zoogonous mirus</i>	11-14 cl 12 som					Wassermann, '12	Verh. Anat. Gesell., 26, p. 47
						Wassermann, '13	Arch. mikr. Anat., 83, Abt. II, p. 1

8. Monogenetics

<i>Gyrodactylus elegans</i>	8 cl (some- times 9)	89	Proph. 89	49 4♂ pron	Karyophytes	Von Ipiolai, '03	Zool. Anz., 28, p. 241.
<i>Gyrodactylus elegans</i>		89	89	4♂ pron	Reduction in 2nd	Katharinet, '04	Zool. Jahrb. Suppl., 7, p. 519
<i>Gyrodactylus elegans</i>	12 spg 12 cl	69	69	69 6♂ pron	Karyomerites	Gille, '14	Arch. Zell., 12, p. 415
<i>Polystomum integerri- mum</i>	ca. 20 cl	109		109		Harkin, '02	Arch. Biol., 18, p. 291
<i>Polystomum integerri- mum</i>	8 cl	89	89	49		Goldschmidt, '02	Zeit. wiss. Zool., 71, p. 349
c. TURBELLARIA							
1. Polyclastidae							
<i>Cyrtogaster papillacea</i>	16 cl	89	89	89		Francotte, '97	Mém. Cour. Acad. Roy. Belgique, 56, p. 5
<i>Eustylocheus ellipticus</i>	20 cl	109	109	109 pron 10♂ pron		Francotte, '98	Arch. Zool. exper. et gen., Ser. III, t. 6, p. 189
<i>Leptopygma tritellaria</i>	16 cl	89	89	89		Van Name, '99	Trans. Conn. Acad. Sc. 10, p. 263
<i>Oligodactylus auritus</i>		89				Francotte, '97	Mém. Cour. Acad. Roy. Belgique, 56, p. 5
<i>Planocera inquilina</i>						Francotte, '97	Mém. Cour. Acad. Roy. Belgique, 56, p. 5
<i>Planocera inquilina</i>		109	109	9-109		Wheeler, '94	Jour. Morph., 9, p. 167
<i>Planocera nebulosa</i>	20 cl	109	109	109 10♂ pron		Patterson and Wieman, '12	Ecol. Bull., 23, p. 271
<i>Prostheocranus vittatus</i>	12 cl	76♂	89	89 6♂ pron		Van Name, '99	Trans. Conn. Acad. Sc. 10, p. 263
						Klinckschmidt, '97	Arch. Naturh. Anat., 46, p. 567

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Prosthecaeus vittatus</i> ...		6 ♀	6 ♀	6 ♀ pron		Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 56, p. 6
<i>Prosthecaeus vittatus</i> ...		6 ♀				Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 189
<i>Prosthecaeus siphunculus</i>	16 cl	8 ♀	8 ♀	8 ♀		Gerard, '01	La Cellule, 18, p. 139
<i>Stylochus pelidium</i>		9 ♀				Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 189
						Gerard, '01	La Cellule, 18, p. 139
<i>2. Rhabdocoela</i>							
<i>Graffia gemellipara</i>	8 cl	4 ♀				Patterson, '12	Biol. Bull., 22, p. 173
<i>Graffia gemellipara</i> , see <i>Paravortex g.</i>	ca. 7 cl				Probably refers to cleavage	Schneider, '33	"Das Ei und Seine Be- fruchtung." Breslau
<i>Mesostomum ehrenbergi</i> .	10 cl			5 ♀	Summer egg	Breslau, '04	Zeit. wiss. Zool., 76, p. 218
<i>Mesostomum ehrenbergi</i> .	10 oog	5 (pairs) ♀	5 ♀	5 ♀ 5 ♂ pron		Von Voss, '14	Arch. Zellf., 12, p. 159
<i>Paravortex cardii</i>	4 cl		4 ♀ (=8 al)	2 ♀ (=4 al)		Halles, '08	C. R. Acad. Sc., 147, p 314
<i>Paravortex gemellipara</i> (<i>Graffia g.</i>).....	8 cl		4 ♀			Halles, '08	Arch. Zool. exper. et gen., Ser. IV, t. 9, p. 429
<i>Polychoerus caudatus</i> ...	31 cl					Ball, '16	Jour. Morph., 27, p. 483
<i>Vortex viridis</i>	4 spk 4 som	2 ♂	2 ♂		Two chroms fuse in 2nd div.	Gardiner, '98	Jour. Morph., 15, p. 73
						Lepeschkin, '10	Biol. Zits. Moscow, 1, p. 104

8. Tricladida

	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in 1st and 2nd metaphase; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Dendrocoelum lacteum...							
Dendrocoelum lacteum...							
Dendrocoelum lacteum, see Planaria lactea	14 spg	8♂ 7♀	8♂			Schleip, '07 Gelei, '13	Zool. Jahrb., 24, p. 129 Arch. Zellf., 11, p. 51
Planaria alpina.....	20-24 spg					Rappeport, '15	Arch. Zellf., 14, p. 1
Planaria goniocephala....	16 spg 16 oog	8♂ 8♀	8♂	8♂		Schleip, '06 Schleip, '07	Zool. Jahrb., 23, p. 357 Zool. Jahrb., 24, p. 129
Planaria lactea (= Dendrocoelum lacteum)...	16 oog	8♀ 8♂	8♂	8♂		Arnold, '09	Arch. Zellf., 3, p. 431
Planaria lactea, see Dendrocoelum lacteum							
Planaria polychroma.....	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in metaphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Planaria simplissima.....	8 spg 6 cl 6 som	3 or 4♂ 3, 4, 6♀	3 or 4♂ 3-4♀		May be two kinds, 3 and 6 reduced	Stevens, '04	Proc. Acad. Nat. Sc. Phila., 56, p. 208
Planaria torva.....	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in metaphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Anz., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
Polycelis nigra.....						Schleip, '07	Zool. Jahrb., 24, p. 129
Procerodes gelachei.....	12 spg 12 som	8♂ 6♂	8♂ 6♂			Böhmig, '07	Arch. Biol., 23, p. 1
Thysanozoon broochi.....	18 spg 18 cl	9♂ 9♀	9♀	9♂ pron 9♀		Van der Stricht, '97 Van der Stricht, '98	Verh. Anat. Ges. 11, p. 92 Arch. Biol. 15, p. 367
Thysanozoon broochi....	18 cl	9♀	9♀			Schockaert, '02 Schockaert, '05	La Cellule, 20, p. 101 La Cellule, 22, p. 1

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	5 spg	3♂	3♂ or 2, 3	2, 3♂	X to pole in 1st or 2nd (3 worms)	Edwards, '10	Science, 31, p. 514 Arch. Zellf., 5, p. 422
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	4 cl	2♀	2♀	2♀ pron		Retsius, '11	Biol. Untersuchungen, 16, p. 21
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	4 cl			2♂ pron		Vejdovsky, '12	König. Böhm. Gesel. Wissen. Prag, p. 1
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	1-8 cl 27♂ (?) emb. 36♀ (?) emb.			1-2♂ pron	Abnormalities in cl. (one animal).	Kautsch, '12	Arch. Entwickl., 35, p. 642
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	5 cl (♂?) 8 cl (♀?) or 4 cl	3♀ or 2	3♀ or 2	3♀ or 2 2 or 3♂ pron	X separate or fused with another chrom	Frolowa, '12	Arch. Zellf., 9, p. 149
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	4 spg 4 oog 4 cl	2♂ 2♀	2♂ 2♀	2♂ 2♀		Fauré Fremiet, '13	Arch. d'Anat. mikr., 15, p. 435
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	4 oog		2♀			Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>		2♀				de Saedeleer, '13	La Cellule, 23, p. 301
<i>Ascaris megalocephala</i> , var. <i>bivalens</i>	2-7 cl	4♀, rarely 5 due to piece from 1 chrom. bro- ken off	4♀, some- times 8, no 1st p. b.; 5 or 6 due to frag- ments	2♀, also sev- eral due to fragmenta- tion	One animal. Dyads instead of tetrads	Geinitz, '15	Arch. Zellf., 13, p. 588
	4 cl (no X) 5 cl (1 X) 6 cl (2 X) 7 cl (3 X) and only 1 p. b. ca. 32♂ emb (48-54) ca. 80♀ emb (58-62)	3♀	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	2♀ (no X) 3♀ (1 X) 4♀ (2 X)	Three animals. X (double) to pole in 1st or 2nd, or di- vides in both. X = 8 in embryos. Rarely X=4 cl		

<i>Ascaris megalocephala</i> , var. <i>trivalens</i> (Zacharias, '12)	3 cl			1♂ (?) pron 2 ♀ (?) pron	Probably crosses biv. X univ. Sometimes small ac- ces. chrom	Zacharias, '12	Biol. Centrall., 32, p. 718
<i>Ascaris megalocephala</i> , var. <i>univalens</i> (Hertwig, '90)	2 cl	1 ♀	1 ♀	1 ♀ 1♂ pron	Chromatin diminution and fragmentation of chroms in som cells	Boveri, '87 Boveri, '88 Boveri, '92 Boveri, '99 Boveri, '04 Boveri, '09	Jen. Zeits., 14, p. 423 (Zellen-Studien I) Jen. Zeits., 15, p. 685 (Zellen-Studien II) Sitz. Gesel. Morph. u. Physiol. München 8, p. 114 Fest. Von Kupffer, p. 383 "Ergeb. Konstitution d. chrom. Substanz d. Zellkerns," Jena Arch. Zellf., 3, p. 181
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl			1♂ pron 1 ♀ pron		Dostojewsky, '88	Anat. Anz., 3, p. 646
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl					Kulschitzky, '88	Arch. mikr. Anat., 31, p. 867
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg 2 oog 2 cl	1♂	1♂	1♂		Hertwig, '90	Arch. mikr. Anat., 36 p. 1
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg 2 oog 2 cl	1♂ 1 ♀	1♂ 1 ♀	1♂ 1 ♀		Schneider, '91	Arch. mikr. Anat., 36 p. 179
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 sex cells					Von Wasmowski, '93	Arch. mikr. Anat., 41, p. 324
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg	1♂	1♂	1♂		Brauer, '93	Arch. mikr. Anat., 42, p. 153
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl	4 el ♀	2 el ♀	1 ♀ 1♂ pron		Herla, '96	Arch. Biol. 13, p. 423
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl			1♂ pron 1 ♀ pron		Carnoy and Lebrun, '97	La Cellule, 13, p. 61
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 sex cells 2 cl				Diminution and of fragmentation of chroms in som cl	Nuesbaum, '02	Arch. mikr. Anat., 59, p. 647

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg	1♂	1♂	1♂		Tretjakoff, '05	Arch. mikr. Anat., 65, p. 383
<i>Ascaris megalocephala</i> , var. <i>univalens</i>		1♀				Bonnevie, '08	Arch. Zellf., 2, p. 201
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	3 cl				Small chrom due to fragmentation or a sex chrom (in 1 or 2 eggs)	Boring, '09	Arch. Zellf., 4, p. 120
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	3 cl				X in ♂. One worm	Edwards, '10	Arch. Zellf., 5, p. 422
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 oog	1♀ (-4 cl)				Blaukertz, '10	Arch. Zellf., 6, p. 1
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 cl			1♂ pron 1♀ pron		Zacharias, '12	Anat. Anz., 42, p. 353
<i>Ascaris megalocephala</i> , var. <i>univalens</i>				1♂ pron 1♀ pron		Held, '12	Verh. Anat. Gesel., 26, p. 242
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 spg 2 oog	1♀				Fauré Fremiet, '13	Arch. d'Anat. mikr., 15, p. 435
<i>Ascaris megalocephala</i> , var. <i>univalens</i>	2 oog	1♀ 1♂				Reisius, '14	Biol. Untersuchungen, 17, pp. 19 and 30
<i>Ascaris mystax</i> , see <i>A.</i> <i>canis</i>							
<i>Ascaris nigrovirens</i> , see <i>Angiostomum n.</i>							
<i>Ascaris triquetra</i>	22 sex cells					Walton, '16	Jour. Parasitol., 3, p. 39
<i>Coronilla scillicola</i> (or ro- busta).....	8 cl	8♀	4♀	2♀ 4♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
<i>Cucullanus elegans</i>	12 ci					Martini, '03	Zeit. wiss. Zool., 74, p. 501

<i>Filaria papillosa</i>	11♂ cl 12♀ cl	6♀	6♀	5, 6♂ 2♀ Prob. 4♂ pron		Meves, '16	Arch. mikr. Anat., 87, II, p. 12
<i>Filaroides mustelorum</i>	16 cl	8♀	4♀			Carnoy, '96	La Cellule, 3, pp. 1 and 63
<i>Heterakis</i> sp?.....	9 spg	4♂ 5♀	4, 5♂ 5♀	4, 5♂ 5♀	X to pole in 1st	Boveri, '09	Arch. Zellf., 4, p. 136
<i>Heterakis dispar</i>	9 spg 10 oog	5♂ 5♀	4, 5♂ 5♀	4, 5♂ 5♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Heterakis infixa</i>	12 oog	1♂ 5♀	4, 5♂ 5♀		X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Heterakis vesicularis</i>	9 spg 10 oog	5♂ 5♀	4, 5♂ 5♀	4, 5♂ 5♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Ophiotostomum mucronatum</i>	12 cl	6 (double) ♀	6 (double) ♀	6♀ 4+♂ pron		Carnoy, '96	La Cellule, 3, pp. 1 and 63
<i>Oxyuris ambigua</i>	3-47 spg	1-3♀	1-3♀	1-3♀		Löwenthal, '89 Löwenthal, '90	Inter. Monats. Anat. u. Physiol. 6, p. 364 Inter. Monats. Anat. u. Physiol., 7, p. 375
<i>Rhabditis</i> sp?.....	18 cl 18 som	10♂ (-8 biv. +2 univ.) Rarely 9 biv. no univ. 18♀	10♂	9♂ 18♀	Only 1 p. b. Sperm degenerate in egg, really pa. XY to poles in 2nd ♂. Occasionally one sex chrom dis- carded in Rest- körper	Kruger, '12 Kruger, '13	Zool. Ans., 40, p. 223 Zeit. wiss. Zool., 105, p. 87
<i>Rhabditis</i> sp?.....	11 cl (♂?) 12 cl (♀?)	6♂ 6♀	5, 6♂ 6♀	5, 6♂ (sperm with ♀ not functional) 6♀	Separate generations X to pole in 1st	Boveri, '11	Verh. phys. med. Ge- sell. Würzburg, 41, p. 83
<i>Rhabditis</i> sp?.....	15 cl 15 oob 15 abd 12 oog 12 som ♀	5+2 X♂ 6♀	5+2 X♂ 6♀	5, 6, 7♂ (sperm with 7 degenerate) 6♀	Hermaphroditic generation 2 X to pole in 2nd or X to each pole		
<i>Rhabditis</i> sp?.....	15 cl	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀	X to pole in 1st or 2nd	Kühn, '13	Arch. mikr. Anat., 83, Abt. II, p. 191
<i>Solerostronchium</i> (Stron- gylus) edentatum.....	11 spg 12 oog	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀			
<i>Solerostronchium equinum</i>							
<i>Solerostronchium vulgare</i>							

VIII. NEMATHELMINTHES—Continued

SPECIES	DIPLOID AND PARHENO- GENETIC	1ST -OTTE	2ND -OTTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Spioptera strumosa</i>	12 cl	8♀	4♀	2♀ 4♂ pron		Carnoy, '86	La Cellule, 3, pp. 1 and 63
<i>Strongylus</i> , see <i>Sclero-</i> <i>stomum</i>							
<i>Strongylus flaria</i>	13 spg 12 oog 12 cl	6♂ 6♀	6♂	6♂ 6♀		Struckmann, '06	Zool. Jahrb., 22, p. 577
<i>Strongylus paradoxus</i>		6♀				Struckmann, '06	Zool. Jahrb., 22, p. 577
<i>Strongylus paradoxus</i>	11 spg 12 oog 11 cl♂ 12 cl♀	6♂ 6♀	5, 6♂ 6♀	5, 6♂ 6♀	X to pole in 1st	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Strongylus tenuis</i>		6♂	6♂	5, 6♂	X to pole in 2nd	Gulick, '11	Arch. Zellf., 6, p. 339
<i>Strongylus tetraoanthus</i> ..		6♀				Meyer, '06	Jen. Zeits., 22, p. 391

IX. NEMERTINEA

a. DITYARIA

<i>Tetrastemma vermiculus</i> .		4♀	2♀	2♀		Lebedinsky, '97 Lebedinsky, '97	Arch. mikr. Anat., 49, p. 503 Biol. Centralb., 17, p. 113
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b. TRITYARIA

<i>Cerebratulus lacteus</i>			5♀ (in p. b.)			C. B. Wilson, '00	Q. J. M. S., 43, p. 97
<i>Cerebratulus lacteus</i>	36 or 38 cl	18 or 19♀				Yatsu, '07 Yatsu, '09	Biol. Bull., 13, p. 300 Jour. Morph., 20, p. 353
<i>Cerebratulus marginatus</i> .	32 cl	16♀	16♀	16♀		Coe, '99	Zool. Jahrb., 12, p. 425
<i>Cerebratulus marginatus</i> .		16♂	16♀	16♀		Kostanecki '02	Bull. Inter. Acad. Sc. Cracovie 1902, p. 270

<i>Linus gessneria</i>	32 ♀ cl	8 ♀	8 ♀	Arnold '99	Trav. Soc. Imp. Nat. St. Petersburg no. 9, p. 1
<i>Linus lacteus</i>	16 cl	10♂ ♀		Meek '13	Phil. Trans. Roy. Soc. London 203B, p. 1.
<i>Linus ruber</i>		8 ♀ (=32 cl)	8 ♀ 8♂ pron	Nussbaum and Orner, '13	Zeit. wiss. Zool., 107, p. 78
<i>Micrus caeca</i>	32 cl	16 ♀		Coe, '99	Zool. Jahrb., 12, p. 425

X. PLATHELMINTHES

a. CESTODA

<i>Avitellina centripunctata</i>	12-14 ? som	47 ♀		Gough, '11	Q. J. M. S., 56, p. 317
<i>Monstia expansa</i>	} 6-9 ? ♂	6-9 ? ♂		Child, '07	Biol. Bull., 12, pp. 89, 191
<i>Monstia planisina</i> ...				Von Janicki, '07	Zeit. wiss. Zool., 87, p. 685
<i>Taenia serrata</i>		6-16 ♀ (prob. 13-15 ♀)			

b. TREMATODA

1. Digenea

<i>Bilharzia haematobia</i> , see <i>Schistosomum haematobium</i>	20 spg 20 oeg 20 cl	10♂ 10♀		Von Kennitz, '13	Arch. Zellf., 10, p. 470
<i>Brachycoelium salmandrae</i> (= <i>B. crassicoile</i>)...					
<i>Dicrocoelium lanceatum</i> (= <i>Distomum lanceolum</i>).....	20 oeg	10 ♀	10 ♀	Goldschmidt, '08	Arch. Zellf., 1, p. 232
<i>Dicrocoelium lanceatum</i> (= <i>Distomum lanceolum</i>).....	20 spg	10♂	10♂	Dingler, '10	Arch. Zellf., 4, p. 672
<i>Diplodiscus temporatus</i> ...	16 pa cl	16 ♀		Cary, '09	Zool. Jahrb., 28, p. 595
<i>Distomum hepaticum</i> ...		6-8 ♀		Henneguy, '06	Arch. d'Anat. microsc., 9, p. 47
<i>Distomum hepaticum</i> , see <i>Fasciola hepatica</i>			One p. b. for pa.		

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENOGENETIC	1st -CITS	2ND -CITS	-TID	REMARKS	OBSERVER	REFERENCE
<i>Diplostomum laticolatum</i>	13 cl	9 ♀	9 ♀	9 ♀		Levy, '14	Arch. mikr. Anat., 85, Abt. II, p. 125
<i>Dicrocoelium lan- ceatum</i>	8 cl			4♂ pron 4♀ pron		Schubmann, '06	Zool. Jahrb., 21, p. 571
<i>Diplostomum furtivum</i>	23 oeg 12 cl	6 ♀, some each 12 ♀, no reduc- tion	6 ♀	6 ♀		Schellenberg, '11	Arch. Zellf., 6, p. 443
<i>Fasciola hepatica</i>	10 (pairs) spg 10 (pairs) oeg	10 (pairs) ♂ 10 (pairs) ♀	5 (pairs) ♂	5 (pairs) ♂	5 pairs to each pole in 1st div.	Deborne, '11	Arch. Zool. exp. et gen. Ser. V, t. 9, p. 1
<i>Schistosomum haemat- obium</i> (= <i>Bilharzia haemato- matobia</i>)	14 spg	8♂ 8♀	6, 8♂	♂, 3♂	2 X to pole in 1st	Lindner, '14	Arch. Zellf., 12, p. 516
<i>Zoogonopus mirus</i>	10 spg 10 oeg 10 cl 10 squam	12-16 ♀	10 ♀	5 ♀ 5♂ pron	Reduction in 2nd div. 5 to each pole	Goldschmidt, '05 Goldschmidt, '08	Zool. Jahrb., 21, p. 607 Arch. Zellf., 2, p. 343
<i>Zoogonopus mirus</i>	20-4 cl 22-26 som	11-13 ♀	11-13 ♀	11-13 ♀ 10-13♂ pron		Schreiner, '08	Skr. Videnak-Selsk. Chemiol., Math. Naturv., 1, no. 6, p. 1
<i>Zoogonopus mirus</i>	12 spg 12-14 cl (prob. 12 cl) 12-14 som	6♂ 6♀	6 ♀	6♂ pron 6♀		Gregoire, '09	La Cellule, 26, p. 243
<i>Zoogonopus mirus</i>	11-14 (prob.) oog 11-14 cl 12 som	6-7 ♀	6 ♀	7♂		Wassermann, '11	Sitz. Gesel. Morph. u. Physiol. München, 27, p. 128
						Wassermann, '12	Verh. Anat. Gesel., 26, p. 47
						Wassermann, '13	Arch. mikr. Anat., 83, Abt. II, p. 1

2. Monogenetics

Species	8 cl (some- times 9)	8 ♀	Proph. 8 ♀	Karyotypes	Reduction in 2nd	References
<i>Gyrodactylus elegans</i> ...		8 ♀	8 ♀		4 ♀ 4 ♂ pron	Von Jepschl, '03 Zool. Anz., 36, p. 241
<i>Gyrodactylus elegans</i> ...		8 ♀	8 ♀		8 ♀ 8 ♂ pron	Katharinet, '04 Zool. Jahrb. Suppl., 7, p. 619
<i>Gyrodactylus elegans</i> ...	12 spg 12 cl	8 ♀	8 ♀			Gille, '14 Arch. Zell., 12, p. 415
<i>Polystomum integerrim-</i> <i>um</i> ...	ca. 20 cl	10 ♀			10 ♀	Wittlin, '02 Arch. Biol., 18, p. 291
<i>Polystomum integerrim-</i> <i>um</i> ...	8 cl	8 ♀	8 ♀		4 ♀	Goldschmidt, '02 Zeit. wiss. Zool., 71, p. 349

3. TURBELLARIA

1. Polychaetes

Species	20 cl	8 ♀	8 ♀	8 ♀	References
<i>Gyrodactylus papilio</i> ...		8 ♀	8 ♀	8 ♀	Francotte, '97 Mém. Cour. Acad. Roy. Belgique, 56, p. 8
<i>Eustylocheus ellipticus</i> ...	20 cl	10 ♀	10 ♀	10 ♀ pron 10 ♂ pron	Francotte, '98 Arch. Zool. exper. et gen., Ser. III, t. 6, p. 149
<i>Leptopliana tritricharia</i> ...	16 cl	8 ♀	8 ♀		Van Name, '99 Trans. Conn. Acad. Sc. 10, p. 283
<i>Oligodactylus avitus</i> ...		8 ♀	8 ♀		Francotte, '97 Mém. Cour. Acad. Roy. Belgique, 56, p. 8
<i>Planocera inquilina</i> ...		10 ♀	10 ♀		Francotte, '97 Mém. Cour. Acad. Roy. Belgique, 56, p. 8
<i>Planocera inquilina</i> ...		10 ♀	10 ♀		Wheeler, '04 Journ. Morph., 9, p. 167
<i>Planocera sabulosa</i> ...	20 cl	10 ♀	10 ♀		Patterson and Wieman, '12 Biol. Bull., 23, p. 271
<i>Prothecarsenus vittatus</i> ...	12 cl	8 ♀	8 ♀		Van Name, '99 Trans. Conn. Acad. Sc. 10, p. 283
					Kiltschowskii, Anat., 48, p. 587

X. PLATHELMINTHES—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Prosthoeceus vittatus</i> ..		6♀	6♀	6♀ pron		Francotte, '97	Mem. Cour. Acad. Roy. Belgique, 56, p. 6
<i>Prosthoeceus vittatus</i> ..		6♀				Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 139
<i>Prothiostomum siphuncula</i>	16 cl	8♀	8♀	8♀		Gerard, '01	La Cellule, 18, p. 139
<i>Stylochus pelidum</i>		9♀				Francotte, '98	Arch. Zool. exp. et gen. Ser. III, t. 6, p. 139
						Gerard, '01	La Cellule, 18, p. 139
<i>s. Rhabdocoela</i>							
<i>Graffilla gemellipara</i>	8 cl	4♀				Patterson, '12	Biol. Bull., 22, p. 173
<i>Graffilla gemellipara</i> , see <i>Paravortex g.</i>	ca. 7 cl				Probably refers to cleavage	Schneider, '83	"Das Ei und Seine Be- fruchtung." Breslau
<i>Mesostomum ehrenbergi</i> .	10 cl			5♀	Summer egg	Breslau, '04	Zeit. wiss. Zool., 76, p. 213
<i>Mesostomum ehrenbergi</i> .	10 oog	5 (pairs) ♀	5♀	5♂ pron		Von Voss, '14	Arch. Zellf., 12, p. 159
<i>Mesostoma ehrenbergi</i>	4 cl		4♀ (-8 al)	2♀ (-4 al)		Halles, '08	C. R. Acad. Sc., 147, p 314
<i>Paravortex cardii</i>						Halles, '08	Arch. Zool. exper. et gen., Ser. IV, t. 9, p. 429
<i>Paravortex gemellipara</i> (<i>Graffilla g.</i>).....	8 cl		4♀			Ball, '16	Jour. Morph., 27, p. 453
<i>Polychoerus caudatus</i>	31 cl					Gardiner, '98	Jour. Morph., 16, p. 73
<i>Vortex viridis</i>	4 spx 4 som	2♂	2♂		Two chroms fuse in 2nd div.	Lepeschkin, '10	Biol. Zeit. Moscow, 1, p. 104

3. *Tricladida*

	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in 1st and 2nd metaphase; 4 to each pole in 1st and 2nd div.		
<i>Dendrocoelum lacteum</i> ...						Mattieson, '04 Mattieson, '04	Zool. Ans., 27, p. 24 Zeit. wiss. Zool., 77, p. 274
<i>Dendrocoelum lacteum</i> ...						Schleip, '07	Zool. Jahrb., 24, p. 129
<i>Dendrocoelum lacteum</i> ...	14 spg	8♂ 7♀	8♂			Golei, '13	Arch. Zellf., 11, p. 51
<i>Dendrocoelum lacteum</i> , see <i>Planaria lactea</i>							
<i>Planaria alpina</i>	20-24 spg					Rappeport, '15	Arch. Zellf., 14, p. 1
<i>Planaria gonoccephala</i>	16 spg 16 oog	8♂ 8♀	8♂	8♂		Schleip, '06 Schleip, '07	Zool. Jahrb., 23, p. 357 Zool. Jahrb., 24, p. 129
<i>Planaria lactea</i> (= <i>Dendrocoelum lacteum</i>)...	16 oog	8 ♀ 8♂	8♂	8♂		Arnold, '09	Arch. Zellf., 3, p. 431
<i>Planaria lactea</i> , see <i>Dendrocoelum lacteum</i>							
<i>Planaria polychroma</i>	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in metaphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Ans., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
<i>Planaria simplissima</i>	8 spg 6 cl 6 som	3 or 4♂ 3, 4, 6♀	3 or 4♂ 3-4 ♀		May be two kinds, 3 and 6 reduced	Stevens, '04	Proc. Acad. Nat. So. Phila., 56, p. 208
<i>Planaria torva</i>	16 cl	8 ♀ (4-8), 4 in prophase	8 ♀ (4 in prophase)	4 ♀	No. doubled in metaphases; 4 to each pole in 1st and 2nd div.	Mattieson, '04 Mattieson, '04	Zool. Ans., 27, p. 34 Zeit. wiss. Zool., 77, p. 274
<i>Polycelis nigra</i>						Schleip, '07	Zool. Jahrb., 24, p. 129
<i>Procerodes gelaschei</i>	12 spg 12 som	8♂ 6♂	8♂ 6♂			Böhmig, '07	Arch. Biol., 23, p. 1
<i>Thysanozoon broochi</i>	18 spg 18 cl	9♂ 9♀	9 ♀	9♂ pron 9♀		Van der Stricht, '97 Van der Stricht, '98	Verh. Anat. Ges. 11, p. 92 Arch. Biol. 15, p. 387
<i>Thysanozoon broochi</i> ...	18 cl	9 ♀	9 ♀			Schockaert, '02 Schockaert, '05	La Cellule, 20, p. 101 La Cellule, 22, p. 1

XI. PORIFERA

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CTTE	2ND -CTTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Clathrina coriacea</i>	ca. 16 collar cells	9-10 ♀				Robertson and Minchin, '10	Q. J. M. S., 56, p. 611
<i>Grantia compressa</i>	8-10 spg 8-10 oog					Dendy, '14	Q. J. M. S., 60, p. 213
<i>Sycandra raphanus</i>	32 cl			16 ♀		Maas, '99	Anat. Ans., 16, p. 290
<i>Sycandra raphanus</i>	8 oog 16 cl	8 ♀	8 ♀	8 ♀		Jørgensen, '09	Arch. Zellf., 4, p. 183

XII. ROTIFERA

<i>Hydatina senta</i>	10 or 12 ♀ pa egg 5 ♂ pa egg 12 winter egg				No. p. b.'s 1 p. b. Prob. 2 p. b.'s	Lessen, '98	La Cellule, 14, p. 419
<i>Hydatina senta</i>	25-25 ♀ pa egg 11-13 ♂ pa egg 14 winter egg (fert)				1 p. b. 2 p. b.'s 2 p. b.'s	Lessen, '98 Whitney, '09	Zool. Ans., 21, p. 617 J. Exp. Zool., 6, p. 127

B. PROTOCHORDATA

I. ACRANIA (CEPHALOCHORDA)

<i>Amphioxus lanceolatus</i> ...		10? ♀			1 count	Van der Stricht, '95	Bull. Acad. roy. Bel- gique, Ser. 3, t. 30, p. 539. Same as
<i>Amphioxus lanceolatus</i> ...	24 cl	10-15 ♀ (prob. 12)				Van der Stricht, '96	Arch. Biol., 14, p. 409
<i>Amphioxus lanceolatus</i> ...	24 oog	12 ♀	12 ♀	12 ♀		Sobotta, '97	Arch. mikr. Anat., 50, p. 15
						Cerfontaine, '05	Acad. roy. Belgique, Cl. de Sciences, '05, p. 643. Same as
						Cerfontaine, '05	Arch. Biol., 22, p. 229

II. UROCHORDA

<i>Ascidia mentula</i>		9 ♀					Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
<i>Ciona intestinalis</i>	18 cl			127 ♀	97 ♂ pron 97 ♀ pron		Boveri, '90	Jen. Zeits., 17, p. 314 (Zellen-Studien III)
<i>Distaplia occidentalis</i>							Bancroft, '90	Bull. Mus. Comp. Zool. Harvard, 35, p. 87
<i>Phallusia mamillata</i>	16 cl (13-16)	8 ♀		8 ♀	87 ♀ (4-9) 8 ♂ pron (8 or 9)		Hill, '95	Rep. Brit. Assoc. Adv. Sci. Ipswich, p. 474
							Hill, '95	Q. J. M. S., 38, p. 315
<i>Stylopis grossularis</i>	4 spg 4 oog	4 ♂ 8 ♀		2 ♂ 4 ♀	1 ♂ 2 ♀	Chroms of sperma- tid probably di- vide in two in fert.	Julia, '93	Bull. sc. Fr. et Bel- gique, 25, p. 93

C. VERTEBRATA

I. AMPHIBIA

a. ANURA

<i>Alytes obstetricans</i>	32 spg	16 ♂					Jansems et Wil- kens, '09	La Cellule, 25, p. 151
<i>Bombinator igneus</i>						Lebrun, '01	La Cellule, 19, p. 315
<i>Bufo calamita</i>		12+ ♀	6-7 ♀				Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, p. 6, t. 101
<i>Bufo lentiginosus</i>	24 spg 24 oog	12 ♂ 12 ♀	12 ♂ 12 ♀	12 ♀			King, '02 King, '07	Anat. Ans., 21, p. 414 Amer. Jour. Anat., 7, p. 345
							King, '08	Jour. Morph., 19, p. 369
<i>Bufo vulgaris</i>		8-10 ♀	8 ♀				Carnoy and Le- brun, '00	La Cellule, 17, p. 199
							Lebrun, '01	La Cellule, 19, p. 315
<i>Bufo vulgaris</i>	18-24 oog						Della Valle, '07	Atti R. Accad. Sc. di Napoli, Ser. 2a, no. 18, vol. 18, p. 1
<i>Bufo vulgaris</i>		8-9 ♀					Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, t. 6, p. 101
<i>Pelodytes punctatus</i>		6 ♀					Bataillon, '10	Arch. Zool. exp. et gen., Ser. V, t. 6, p. 101

I. AMPHIBIA—Continued

SPECIES	DIPLOID AND PARtheno- GENETIC	1st -CTYE	2ND -CTYE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Rana catesbiana</i>	26 oog					Swingle, '17	Biol. Bull., 33, p. 70
<i>Rana esculenta</i> 'Gruener Froesch'.....	24 som					Schottlander, '88	Arch. mikr. Anat., 31, p. 426
<i>Rana esculenta</i>	24 (not stated where)					Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
<i>Rana esculenta</i>	16 spg					Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
<i>Rana esculenta</i>	ca. 25 spg	13♂	12, 13♂	12, 13♂	X to pole in 1st	Levy, '15	Arch. mikr. Anat., 86, II, p. 85
<i>Rana fusca</i> (?).....	24 spg	12♂	12♂	12♂		Vom Rath, '95	Arch. mikr. Anat., 46, p. 168
<i>Rana fusca</i>	12 pa cl	12 ♀				Bataillon, '10	Arch. Zool. exp. et gen. Ser. V, t. 6, p. 101
<i>Rana fusca</i>	20+ pa som				Regulation to nor- mal no.	Brachet, '11	Arch. Biol., 26, p. 337.
<i>Rana pipiens</i>	25 spg 26 oog	13♂	12, 13♂		X to pole in 1st	Swingle, '17	Biol. Bull., 33, p. 70
<i>Rana temporaria</i>		8♂				Bertacchini, '96	Inter. Monats., 13, p. 409
<i>Rana temporaria</i>		8-10 ♀	10 ♀			Carney et Le- brun, '00	La Cellule, 17, p. 199
<i>Rana temporaria</i> (or <i>fusca</i>).....	24 cl ca. 12 pa cl					Lebrun, '01	La Cellule, 19, p. 315
<i>Rana</i> 'Froesch'.....	16 som				Leucocytes	Levy, '13	Arch. mikr. Anat., 82, II, p. 65
'Grenouille'.....	12 (pairs) som 6 pa som			6♂ 6♀		Dekhuysen, '91	Anat. Anz., 6, p. 220
'Leopard frog'.....	20+ pa spg				Adult pa frog. Ob- servation of Gold- schmidt	Dehorne, '10	C. R. Acad. Sc. Paris, 160, p. 1451
						Dehorne, '11	C. R. Acad. Sc. Paris, 162, p. 1123
						Loeb, '18	Proc. Nat. Acad. Sc., 4, p. 60

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I. AMPHIBIA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1st -CTE	2nd -CTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Triton cristatus</i>	18-24 spg	12♂	12♂			Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
<i>Triton cristatus</i>	24 spg					Meek, '13	Phil. Trans. Roy. Soc. London, 203B, p. 1
<i>Triton palmatus</i>	18-24 spg					Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
<i>Triton punctatus</i>	ca. 12-16 som					Reitzius, '81	Biol. Untersuchungen, '81, p. 108
<i>Triton punctatus</i>	24 spg	12♂ 12♀	12♂ 12♀			Janssens, '00 Janssens, '01 Janssens, '02 Janssens, '04	Anat. Anz., 17, p. 520 La Cellule, 19, p. 5 Anat. Anz., 21, p. 129 Anat. Anz., 24, p. 648
<i>Triton taeniatus</i>		12-14♀				Born, '94	Arch. mikr. Anat., 43, p. 1
<i>Triton taeniatus</i>		12♀	12♀			Carnoy et Le- brun, '99	La Cellule, 16, p. 203
<i>Triton vulgaris</i>	18-24 spg					Champy, '13	Arch. Zool. exp. et gen. 52, p. 13
<i>Triton vulgaris</i>	12 pa som				Sperm destroyed with radium	Hertwig, O., '13	Arch. mikr. Anat., 82 II, p. 1
<i>Triton</i>	20 + som (prob. 24)					Rabl, '85	Morph. Jahrb., 10, p. 214
<i>Triton sp.</i>	24 spg	12 (gemini)♂				Moore and Em- bleton, '05 Moore and Ar- nold, '05	Proc. Roy. Soc., Lon- don, 77, p. 555 Proc. Roy. Soc., Lon- don, 77, p. 593

II. AVES

a. ANSERES

<i>Anas boschas</i>							
<i>Aythya ferina</i>							
<i>Carina moschata</i>							
<i>Lamprolaima sponsa</i>							
<i>Mareca penelope</i>							
	ca. 16 spg.	8♂	8♂			Schöneberg, '13	Arch. mikr. Anat., 83, Abt. II, p. 324

b. COLUMBAE

Columba livia domestica	16 spg	8♂	4♂ (occasionally 8)	8 sperm nucleus	Second pairing of chroms before 2nd div.	Guyer, '00 Guyer, '02 Harper, '04 Smith, '12 Guyer, '00 Guyer, '02	Dissertation Univ. Chicago Univ. Cincinnati Bull. 21, Ser. II, vol. II, p. 1 Amer. Jour. Anat., 3, p. 349 Q. J. M. S., 53, p. 169 Dissertation Univ. Chicago Univ. Cincinnati Bull. 21, Ser. II, vol. II, p. 1
Columba livia domestica	16 cl	8♀	8♀	8 sperm nucleus			
Columba 'Pigeon'.....	ca. 16 spg	8♂	4♂				
Turtur risorius.....	16 spg	8♂	4 (occas. 8) ♂		Second pairing of chroms before 2nd div.		

c. GALLINAE

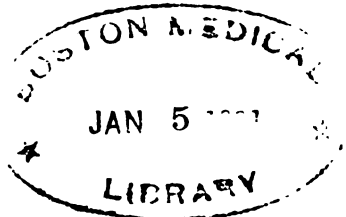
Gallus domesticus.....	18 spg 18♂ som	8-16 (pairs) ♀ (prob. 12)	4, 5♂ (fusion in pairs, may be incomplete giving 6, 7, etc.)	4, 5♂. Those with 4 prob. degenerate	X to pole in 1st. X=2 elements in spg and ♂ som; X=1 element in oog and ♀ som. From correction in '16	Loyes, '06 Sonnenbrodt, '08 Guyer, '09 Guyer, '16	Arch. d'Anat. micros. 8, p. 239 Arch. mikr. Anat., 72, p. 415 Anat. Ans., 34, p. 573 Biol. Bull., 31, p. 221
Gallus domesticus.....	127 ps cl 13 som	9♂	4, 5♂ (fusion in pairs, may be incomplete giving 6, 7, etc.)	4, 5♂. Those with 4 prob. degenerate	Prob. no reduction	Lecaillon, '10 Lecaillon, '10	C. R. Soc. Biol., 69, p. 34 Arch. d'anat. micros., 12, p. 511
Gallus 'Gold Campine fowl'....	19-20 spg	8-10♂			Clumping in 2nd spc.	Cutler, '18	Jour. Genetics, 7, p. 155
Numida meleagris dom. (=domestic guinea)....	17 spg	9♂	8, 9♂	4, 5♂ (bivalent) (few with 6)	X to pole in 1st. Second pairing of chroms before 2nd div.	Guyer, '09	Anat. Ans., 34, p. 502
Phasianus 'Pheasant'.....	20-22 spg	10-11 ♂	5-6 ♂		Secondary pairing and fusion in 2nd div., forming 1-8 masses	Cutler, '18	Jour. Genetics, 7, p. 15

<i>Vesperugo</i> 'Bat'.....	24 spg (at least)	15-22 ♀	18-24 ♀	'Heterochromosomes'	Jordan, '12	Anat. Ans., 40, p. 513
<i>Vesperugo serotinus</i>					Athias, '12	Arch. R. Inst. Bacter. Cam. Vet. Lisbonne, 3, p. 287
c. EDENTATA						
<i>Tatin novemcinctum</i> (= 9-banded Armadillo).....	21? spg 23 oeg	16 ♀ (14-19)			Newman and Paterson, '10 Newman, '12	Jour. Morph., 21, p. 359 Biol. Bull., 23, p. 100
d. MARSUPIALIA						
<i>Didelphys aurita</i>		127 ♀	127 ♀		Hill, '18	Q. J. M. S., 63, p. 91
<i>Didelphys virginiana</i> (= Oposum).....	17 spg 17 som	9 ♂	4, 5♂ (-8, 9, univalents) 4, 5♂ (-8, 9, univalents)	X to pole in 1st. Second pairing of chroms after 1st div.	Jordan, '11	Arch. Zellf., 7, p. 41
<i>Marupialia</i> 'Beuteltiere'.....			4 (often) ♂		Von Bardeleben, '98 Benda, '06	Jen. Zeits., 24, p. 475 Semon Zool. Forsch. Australia u. Malay Archipel., p. 439
<i>Perameles</i> <i>Phalangeria</i>		8 ♂				
e. MONOTREMATA						
<i>Echidna</i> <i>Ornithorhynchus</i>		8-12 ♂			Benda, '06	Semon Zool. Forsch. Australia u. Malay Archipel., p. 415
f. PRIMATES						
<i>Homo sapiens</i> 'Menech'.....	22-28 som (prob. 24)			Cornea	Flemming, '83 Flemming, '98	Arch. mikr. Anat., 20, p. 1 Anat. Ans., 14, p. 171
'Menech'.....	18-40 som			'Normal tissue'	Hansemann, '91	Virch. Arch., 123, p. 356

III. MAMMALIA—Continued

SPECIES	DIPLOID AND PARTHENO- GENETIC	1ST CYTE	2ND CYTE	YID	REMARKS	OBSERVER	REFERENCE
Homo sapiens—contin- ued							
'Mensch'.....	8 spg (= 16 cl)	8♂	4♂	4♂	3 men, 21, 39 and 46 yrs. old	Von Bardeleben, '92 Von Bardeleben, '97 Von Bardeleben, '98	Verh. Anat. Gesell. Wien, p. 202 Arch. Anat. u. Phys. (Anat. Abt) Suppl., p. 193 Jen. Zeits., 24, p. 475
'Man'.....		18♂ (15-19)			Man 54 yrs. old	Wilcox, '00	Anat. Anz., 17, p. 316
'Mensch'.....	32?				Material not de- scribed	Fick, '06	Arch. Anat. u. Phys- iol. (Anat. Abt) Suppl., p. 179
'Man (Homo)'.....	32 spg	16♂ (gemini)				Moore and Ar- nold, '05 Moore and Walk- er, '06	Proc. Roy. Soc. Lon- don, 77B, p. 563 Univ. Liverpool Re- ports, 106, p. 1
'L'homme'.....	ca. 24 spg	12♂			Material not de- scribed	Dueberg, '06	Anat. Anz., 23, p. 475
'Man'.....	22 spg	12♂, few have 14	5, 7♂ (= 16, 12 univalents)	5, 7♂ (= 10, 12 univalents)	Negro 30 yrs. old 2X to pole in 1st. Second pairing of chroms after 1st div.	Guyer, '10	Biol. Bull., 19, p. 219
'L'homme'.....	24 som	ca. 12♂	ca. 18+♂			Branca, '10 Branca, '11 Branca, '12	C. R. Assoc. Anat., 12, p. 6 Bibl. Anat., 21, p. 233 C. R. Assoc. Anat.
'Mensch'.....		ca. 12♂			Man 23 yrs. old. No X chrom	Guthers, '12	Arch. mikr. Anat., 79, (2), p. 79
'L'homme'.....	47 spg (46-49) 48 oog	24♂ (23-25)	23, 24♂	23, 24♂	♂ count from 4 men 21, 23, 25, 41 yrs. old. ♀ count from 4 mo. em- bryo. X to pole in 1st	Von Winiwarter, '12	Arch. Biol., 27, p. 91
'Man'.....		12♂	10, 11 or 12♂	10, 11 or 12♂	2 X to pole in 1st or 2nd. Each di- vides once. Negro aged 50	Montgomery, '12	Jour. Acad. Nat. Sc., Phila., 15, p. 1

	24 spg 33-38 som, mostly 34	12♂	12♂	12♂	12♂	12♂	From 9 mm. em- bryo, also negro and white (age 37) adults. XY to poles in 2nd Double X ?	Wieman, '13 Wieman, '17 Jordan, '14	Amer Jour. Anat., 14, p. 461 Amer Jour. Anat., 21, p. 1 Carneg. Inst. Pub. 182, p. 165
'Man'.....		12♂	12♂	12♂	12♂	12♂			
'Man'.....		12+♂							
9. RODENTIA									
Cavia	16 spg					8♂		Von Bardeleben, '92	Verh. Anat. Gesell., '92, p. 202
'Meerschweinchen'.....								Flemming, '98	Anat. Anz., 14, p. 171
'Meerschweinchen'.....	Prob. 24 som							Moore and Walk- er, '06	Liverpool Univ. Rep., '06, p. 1
'Guinea-pig'.....	32 spg	16♂ (gemini)	16♂				XY to poles in 1st	Stevens, '11	Biol. Bull., 21, p. 155
'Guinea-pig'.....	56? spg	28♂	24-28 ♀					Athias, '12	Arch. R. Inst. Bacter. Cam. Pest. Liabonne, 3, p. 287
Cavia porcellus.....								Lams, '13	Arch. Biol., 28, p. 229
'Cobaye'.....	16 som	8 ♀	8 ♀			8 ♀		Athias, '09	Anat. Anz., 34, p. 1
Eliomys quercinus.....		16 ♀	16 (10-16) ♀					Athias, '12	Arch. R. Inst. Bacter. Cam. Pest. Liabonne, 3, p. 287
Lepus	24? som							Flemming, '98	Anat. Anz., 14, p. 171
'Kaninchen'.....								Von Winiwarter, '00	Arch. Biol., 16, p. 695
'Lapin'.....	41-43 oog 38-46 som (mostly 42)	10-12 ♀ (from Honore)						Von Winiwarter, '01	Arch. Biol., 17, p. 33
'Rabbit'.....	28-36 spg	14-18♂						Barrat, '07	Proc. Roy. Soc. Lon- don, 76B, p. 372
'Rabbit'.....	22 spg	12♂ (=11)	11♂				XY to poles in 1st. XY sometimes double in 1st	Bachhuber, '16	Biol. Bull., 30, p. 294
Microtus incertus.....		28-34 ♀	28-34 ♀					Athias, '12	Arch. R. Inst. Bacter. Cam. Pest. Liabonne, 3, p. 237



III. MAMMALIA—Continued

SPECIES	DIPLOID AND PANTHEROGENETIC	1ST OTTE	2ND OTTE	TID	REMARKS	OBSERVER	REFERENCE
<i>Mus decumanus</i> (see also <i>Mus rattus</i>) 'Rat'.....	32 spg	16♂ (gemini)			Earlier accounts of Moore corrected in '05	Moore, '93 Moore, '94 Moore and Arnold, '05 Moore and Walker, '06	Anat. Anz., 8, p. 583 Intern. Monatschr., 11, p. 129 Proc. Roy. Soc., London, 77B, p. 583 Univ. Liverpool Reports, '06, p. 1
'Ratte'.....		12♂ (3-12)				Lenhossék, '98	Arch. mikr. Anat., 51, p. 215
'Wanderratte'.....	167 spg	8♂	8♂ (3-16)			Von Ebner, '99 Von Ebner, '02	Sitz. Ber. d. k. Akad. Wissen. Wien, 108 (3), p. 439 Kölliker's 'Geweblehre des Menschen,' III
'Rat'.....	20-30 spg	ca. 12♂				Regaud, '01 Regaud, '01 Regaud, '09	C. R. Soc. Biol., 53, p. 406 d'Anat. micros., 4, p. 231 Arch. d'Anat. micros., 11, p. 291
'Mus decumanus var. albinus'.....	24 som (prob)	12♂ (prob)	12♂ (prob)			Duesberg, '08	Arch. Zellf., 1, p. 399
'Weisse Ratte'.....		16♀ (10-20)	167♀ (3-16)			Sobotta u. Burkhard, '10	Anat. Hefte, 42, p. 433
'Mus decumanus albinus'.....	More than 24 spg	16♂	Prob. 16♂			Van Hoof, '11	La Cellule, 27, p. 289
<i>Mus musculus</i> 'Mus musculus, var. blanche et noire'.....	207 cl	20♀	20♀			Tafani, '89	Atti R. Accad. Lincei, Rendiconti, Ser. 4, Vol. 5, p. 119 (also in Pub. Inst. St. Sup. Firenze, Med-chirurg. = Arch. Anat. norm. e path., 5, p. 1
'Maus'.....		16♂				Hermann, '80	Arch. mikr. Anat., 34, p. 58

'Grau Maus'	247 oog	6 groups of 49			Earlier accounts corrected in '07	Holl, '93 Holl, '93	Verh. Anat. Gesell. Göttingen, '98, p. 122 Sitz. Ber. d. k. Akad. Wiss. Wien, 102 (3), p. 249
'Maus, weisse, grau und Taub'	30+cl	189 (10-19)	1679			Sobotta, '93 Sobotta, '95 Sobotta, '07 Sobotta, '08	Verh. Anat. Gesell. Göttingen, '92, p. 111 Arch. mikr. Anat., 46, p. 15 Anat. Hefte, 35, p. 493 Verh. phys-med. Gesell. Würzburg, 35, p. 241
'Souris blanche'	12 spg (10-12)	12♂ (double). Some cells 10 (double)	12♂ (single). Some cells 16	6♂. Some cells 8		Lukianow, '98	Arch. Sc. Biol. St. Petersburg, 6, p. 285
'Mause'	24 spg					Moore and Arnold, '05 Moore and Walker, '06	Proc. Roy. Soc. London, 77B, p. 363 Univ. Liverpool Report, '06, p. 1
'Mus musculus'		129	129			Gerlach, '06	'Über die Bildung der Richtungsorgane bei Mus musculus', Wiesbaden
'Mus musculus=souris blanche'		129 (12-15)	129	129		Lams et Doorme, '07	Arch. Biol., 23, p. 250
'Mus musculus var. alba'		89	89			Malininos, '07	Arch. mikr. Anat., 70, p. 877
'White mouse'		129 (12-24 due to precocious division)	129 (=24 univ)	129		Coe and Kirkham, '07 Kirkham, '07 Kirkham, '08	Science, 35, p. 778 Biol. Bull., 12, p. 259 Trans. Connecticut Acad. Arts and Sc., 12, p. 66
'Mouse, white, black and hybrid whiteX gray'		209	209	209		Long, '08 Long and Mark, '11 Kingsley, '14	Science, 27, p. 443 Carnegie Institute Pub., 142, p. 1 Biol. Bull., 27, p. 240
'White mouse'		12-249	12-309			Yocum, '17	Univ. California Pub., 16, p. 371
'House mouse'		20♂	20♂	19, 20♂	X to pole in 2nd		

III. MAMMALIA—Continued

SPECIES	DIPLOID AND PARHENO- GENETIC	1st -CYTE	2ND -CYTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Mus norvegicus albinus</i> (=white rat).....	40 (pachytene threads of oocyte)					Pratt and Long, '17	Jour. Morph., 29, p. 441
<i>Mus norvegicus albinus</i> ..	37 spg 37♂ som	19♂	18, 19♂		X to pole in 1st	Allen, '18	Jour. Morph., 31, p. 133
<i>Mus rattus</i> , see also <i>Mus</i> <i>decumanus</i>							
' <i>Mus rattus albus</i> '.....		8♀	8♀			Melissinos, '07	Arch. mikr. Anat., 70, p. 577
' <i>Mus rattus albinus</i> '.....	More than 24 spg	16♂	Prob. 16♂			Van Hoof, '11	La Cellule, 27, p. 239
<i>Sciurus</i> ' <i>gambelii</i> '.....	24 + som	ca. 16♂				Van Mollé, '07	La Cellule, 24, p. 257
A. UNGULATA							
<i>Bos</i> ' <i>Stier</i> '.....	16 spg			8♂		Van Bardeleben, '92	Verh. Anat. Gesell., '92, p. 202
' <i>Taureau</i> '.....	24 spg (20-25)	12♂	12♂			Schoenfeld, '02	Arch. Biol., 18, p. 1
' <i>Taureau</i> '.....	Prob. 24 spg (20-24)	12♂				Van Hoof, '13	La Cellule, 30, p. 7
<i>Equus</i> ' <i>Pferde</i> '.....		10-16♂				Kirillow, '12	Arch. mikr. Anat., 70, II, p. 125
' <i>Horse</i> '.....	37 spg	19♂	9, 10♂ (quad- rivalent)	9, 10♂ (biva- lent)	X to pole in 1st. Chrom. pair in tel- ophase of 1st div.	Wodsedalek, '14	Biol. Bull., 27, p. 295
' <i>Mule</i> '.....	51 spg	34-49♂ (mostly 40- 45) some univalent			X. Cells disinte- grate, no 2nd spc	Wodsedalek, '16	Biol. Bull., 30, p. 1-38

<i>Sus</i> 'Pig'	18 spg 18 som ♂ 20 oeg 20 som ♀, sometimes 10 by pair- ing	10♂	8, 10♂	4, 6♂ (auto- somes biva- lent)	2 X to pole in 1st	Wiededalek, '13 Wiededalek, '13	Science, 38, p. 30 Biol. Bul., 25, p. 8
<i>Sus scrofa</i>	40 spg (1 giant cell ♀) 40-58 som (1 cell ♀)	20♂			Variation in no. due to fragmentation	Hance, '17 Hance, '18	Jour. Morph., 30, p. 155 Biol. Bul., 35, p. 33

IV. PISCES							
a. CYCLOSTOMATA							
<i>Bdellostoma burgeri</i>	487 spg					Schreiner, '08	Arch. Zell., 1, p. 152
<i>Myxine glutinosa</i>	ca. 50 som					Retzius, '90	Verh. d. biol. Vereins Stockholm, 2, p. 80
<i>Myxine glutinosa</i>	ca. 52 spg ca. 52 som	26♂ (possibly 27)	26♂			Schreiner, '04 Schreiner, '04	Anat. Ans., 24, p. 561 Arch. Biol., 21, p. 153

b. DIPNOI							
<i>Lepidosteus paradoxus</i>	Prob 38 som (34-37)					Murray, '06	Anat. Ans., 28, p. 203
<i>Lepidosteus paradoxus</i>	38 som	19♂ gemini				Agar, '11 Agar, '12	Q. J. M. S., 57, p. 1 Q. J. M. S., 58, p. 285

c. ELASMOBRANCHII							
<i>Pristiurus melanostomus</i>		30-50 ♀				Kastchenko, '90	Zeit. wiss. Zool., 50, p. 428
<i>Pristiurus</i>	ca. 36 spg 30-36 oeg 30-36 som	ca. 18 ♀	ca. 18 ♀	ca. 18 ♀		Ruckert, '92	Anat. Ans., 7, p. 107
<i>Pristiurus</i>	24 spg	12♂	12♂	12♂		Moore, '96 Farner and Moore, '04	Q. J. M. S., 38, p. 275 Q. J. M. S., 48, p. 489
<i>Raja macrorhynchus</i> <i>Raja maculata</i>						Kastchenko, '90	Zeit. wiss. Zool., 50, p. 428
<i>Scyllium canicula</i>		30-50 ♀					

IV. PISCES—Continued

SPECIES	DIPLOID AND PARHENO- GENETIC	1ST -CTTS	2ND -CTTS	TUD	REMARKS	OBSERVER	REFERENCE
<i>Scyllium canicula</i> catulus	24 spg	12♂	12♂	12♂		Moore, '04 Moore, '95 Farmer and Moore, '04	Anat. Anz., 9, p. 547 Q. J. M. S., 38, p. 275 Q. J. M. S., 48, p. 489
<i>Scyllium canicula</i>		20-24♂	14-16♂			Rawits, '90	Arch. mikr. Anat., 53, p. 19
<i>Scyllium canicula</i>		17-19♀				Cerruti, '08	Atti real. Accad. Sc. fis. e math., Napoli, II s, vol. 13
<i>Spinax niger</i>	60-70 spg	30-50♀				Schreiner, '07	Arch. Biol. 22, p. 419
<i>Torpedo ocellata</i>						Kasatschenko, '90	Zeit. wiss. Zool., 50, p. 428
<i>Torpedo</i>	24 spg	12♂	12♂	12♂		Moore, '95 Farmer and Moore, '04	Q. J. M. S., 38, p. 275 Q. J. M. S., 48, p. 489

d. TELEOSTII							
<i>Osteolaobranchius adspersus</i> ...	38-48 cl					Pinney, '18	Jour. Morph., 31, p. 225
<i>Fundulus heteroclitus</i> ...	36 cl					Moenkhaus, '04	Amer. Jour. Anat., 8, p. 29
<i>Fundulus heteroclitus</i> ...	45 cl					Pinney, '18	Jour. Morph., 31, p. 225
<i>Menidia notata</i>	36 cl					Moenkhaus, '04	Amer. Jour. Anat., 3, p. 29
<i>Salmo fario</i> (= 'Forelle')..		ca. 12♀	ca. 12♀	ca. 12♀		Böhm, '91	Sitz. Gesell. Morph. u. Physiol. München, 7, p. 63
'Forelle'.....	12 cl (prob.)				Sperm treated with radium. Not pa	Oppermann, '13	Arch. mikr. Anat., 83, Abt II, p. 307
<i>Trutta fario</i> (= 'Forelle')	24 cl	12♀	12♀	12♀		Behrens, '98	Anat. Hefte, 10, p. 227
<i>Trutta lacustris</i>		At least 24♀	At least 24♀			Blanc, '04	Ber. Naturf. Gesell. Freiburg, 8, p. 163 (= Festschr. Weismann)

V. REPTILIA
a. CHELONIA

SPECIES	DIPLOID AND PARACHNO- GENETIC	1ST -CTE	2ND -CTE	-TID	REMARKS	OBSERVER	REFERENCE
<i>Chrysemis marginata</i>		17♂			X to pole in 1st	Jordan, '14	Science, 39, p. 438
<i>Cistudo carolina</i>		16♂				Jordan, '14	Science, 39, p. 438

b. LACERTILIA

<i>Anguis fragilis</i> 'Orvet'.....		12♀				Loyes, '05	Arch. de l'Anat. mikr., 8, p. 69
<i>Anguis fragilis</i>	367 som	187♀				Trinci, '08	Mem. R. Acad. Sc. Bologna, Ser. VI, vol. 8, p. 167
<i>Lacerta agilis</i> <i>viridis</i>	Prob 24 spg (20-28)	Prob 12♂ (10-15)				Tallyaniszky, '97	Math. u. Naturw. Ber. Hungarn, 13, p. 303
<i>Lacerta stirpium</i>	24 oog	8-12♀				Loyes, '05	Arch. de l'Anat. mikr., 8, p. 69

III. HISTORICAL AND CRITICAL

The first attempt to count chromosomes in the Metazoa was made in 1878 by Selenka, in his "Befruchtung des Eies von Toxopneustes." He gave the number in the cleavage cells as varying between 14 and 24, a rather wide range and not very close to the mark (36). Retzius² in 1881 gave the number for Triton punctatus as between 12 and 16 in the somatic cells, also somewhat far from correct (24). The next observation, by Flemming³ on the salamander in 1882, was accurate and correct, and to him therefore belongs the credit of establishing a definite number of chromosomes for a definite species. He was also the first to attempt a count on human cells, given in the same publication. Very shortly after this, Strassburger,⁴ '82, gave definite and correct numbers for several species of plants. Then came three papers giving the chromosome numbers in *Ascaris*, Anton Schneider's 'Das Ei und seine Befruchtung' in 1883, Nussbaum's⁵ paper in 1884 and the very thorough and brilliant work of Van Beneden⁶ which was published in 1883, although it did not appear till April 1884. Soon after, still in the '80's, came Carnoy's and Boveri's papers on the nematodes and other works on the nematodes, molluscs and vertebrates. Since then, chromosome counts have been made by many observers on about 960 different species of animals.

Several lists of chromosome numbers have appeared previously, the first by Wilson in 'The Cell' in 1900, a partial list which included about fifty species of animals and a few plants. In 1905, Enriques⁷ gave an incomplete list of numbers in animals, expressing the different numbers in mathematical formulae, as powers of 2 and 3. Montgomery⁸ in 1906 gave a list which was supposed to be very nearly complete, but there are many omissions and a good many inaccuracies in the list. Montgomery's

² G. Retzius. 1881. Biol. Untersuchungen, p. 109.

³ W. Flemming. 1882. Arch. mikr. Anat., 20, p. 1.

⁴ E. Strasburger. 1882. Arch. mikr. Anat., 21.

⁵ M. Nussbaum. 1884. Arch. mikr. Anat., 23, p. 155.

⁶ E. Van Beneden. 1883. Arch. de Biol., 4, p. 265.

⁷ Paolo Enriques. 1905. Archivio di Fisiologia, 2, p. 258.

⁸ T. H. Montgomery. 1906. Trans. Amer. Philos. Soc., 21, p. 97-162.

general conclusion was that chromosome number should be considered as an important factor in taxonomy and that animals having widely different numbers should be placed in different genera. McClung has also been a strong advocate of the value of chromosome numbers in taxonomy. Della Valle's⁹ list in 1909 is of little value, as it is a prejudiced one, given entirely with the object of showing that chromosome numbers are inconstant and of little importance. Two comprehensive lists of chromosome numbers in plants have appeared recently, Tischler's¹⁰ and Ishikawa's¹¹ in 1916. The latter is exclusively a list of numbers, his general conclusions being reserved for a further publication. Tischler's list is accompanied by able discussions and criticisms, his general conclusion being that it is still too soon to solve any large phylogenetic problems on the basis of chromosome investigations. It may be of interest as a comparison with the work on animals to give some of his statements concerning numbers in plants. The Asco- and Basidiomycetes have very small numbers, the mosses and Gymnosperms in general small numbers, whereas the Algae, Pteridophytes and Angiosperms have species with both small and large numbers. The Magnoliaceae and Nymphaeaceae (Angiosperms) and the Ophioglossaceae, Equisetaceae and Lycopodiales (Pteridophytes) have very high numbers, although not a great many species have been studied cytologically. Finally Winge¹² in 1917 has given an additional list in plants and has concluded from that and from Tischler's list that the numbers in related species are in arithmetical progression,—e. g. the chrysanthemums with 9, 18, 27, 36, and 45,—these arising by hybridization of species with like numbers; and that in general numbers occur in factors of 2 and 3 (an idea similar to that of Enriques), the numbers 8 (2.2.2) and 12 (2.2.3) occurring most frequently.

A cursory survey of Tischler's or Ishikawa's list of numbers in plants and of my own list in animals is sufficient to show that very

⁹ P. Della Valle. 1909. *Archivio Zoologico*, 4, p. 1-177.

¹⁰ G. Tischler. 1916. *Progressus Rei Botanicae*, 5, p. 164-260.

¹¹ Mitsuharu Ishikawa. 1916. *The Botanical Magazine, Tokyo*, 30, p. 404-448.

¹² O. Winge. 1917. *C. R. Travaux du Laboratoire de Carlsberg*, 13.

closely related species may have widely different numbers, and that the numbers in related species are not usually in arithmetical ratio although occasionally they are, especially in plants. It is also apparent that numbers which are resolvable into factors of 2 and 3 are of frequent occurrence, as one would expect since nearly half of the numbers between 2 and 20 (the most frequently occurring numbers) are resolvable into these factors. However, it is equally apparent that other numbers not resolvable into these factors are also of frequent occurrence.

In using the present tabulation for any generalizations or conclusions, several facts must be taken into consideration. Many of the observations recorded are of too early a date to be of much value. Other observations are contradictory and in many cases it is impossible to judge which is correct; this is largely due to difficult material and is especially true for the mammals, where for man the number of chromosomes varies between 8 and 48 (diploid) according to different authorities.

IV. CHROMOSOME NUMBERS

In looking over the fore-going list, there can be no doubt to an unprejudiced mind that the constancy of chromosome numbers for a species is a fact, and that any variation in number for a definite species is an exception to the general rule. Such variations occur regularly in *Notonecta insulata*, *Jamaicana unicolor* and *J. subguttata*, and *Hesperotettix viridis* where two or more chromosomes may be united or separate; in species with supernumeraries (see p. 66); in cases where multiple groups occur (e. g. *Culex pipiens*, *Notonecta*, *Anasa*), and where fragmentation has taken place (e. g. *Ascaris*, pig). A few sporadic variations occur in certain species owing to the lack of conjugation of two univalents (e. g. *Lygaeus turcicus*, *Coenus delius*, *Euschistus*) and a few which have not been explained (e. g. *Trichopepla*, *Lygaeus reclinatus* which is now under investigation). When a range of numbers is given instead of one definite number, it is usually due either to the early date at which the observation was made or to difficult material rendering accurate counting impossible.

There is however a great range of numbers among the different forms of animals. As is well known, there is only one chromosome in the haploid groups of *Ascaris megalocephala univalens*; some species of *Gordius* (Nematode) also are reported as having only one chromosome in the reduced groups and *Styelopsis* (Ascidian) as having only one in the spermatid. Indeed, according to Moore, '93, there is only one chromosome in the oogonia of *Apus* (Phyllopod crustacean). Animals having only two chromosomes in the haploid groups are: *Ascaris megalocephala bivalens*, *Cyclops viridis brevispinosus*, *Pediculopsis graminum* (arachnid), *Icerya purchasi* (Homoptera), *Tetrastemma vermiculus* (Nemertean), *Vortex viridis* and *Paravortex cardii* (Rhabdocoels). At the other end of the series are: two species of *Cambarus* (Decapod), with 104 and 100 (reduced), *Artemia* (Phyllopod) with 84, *Cancer* and *Hippa* (Decapods) with 60, *Astacus* (Decapod) with about 58 and *Nyssia* (Moth) with 56. The number occurring most frequently among the forms investigated is 12; other numbers occurring very frequently are 6, 7, 8, 9, 10, 11 and 16.

There is also often a considerable range in number among different forms belonging to the same class, e. g. *Nematoidea* (1-24 reduced), *Aphidae* (3-20), *Copepoda* (2-17). The classes showing the greatest constancy are the *Acrididae* (Orthoptera) and the *Urodeles* (Amphibia). The *Diptera* and the *Nematodes* have, in general, low numbers whereas the *Decapods* and *Lepidoptera* have high numbers.

A chromosome is really a compound structure, carrying many characters or genes which are themselves the elements of heredity. However genes may arise, it is conceivable that in some cases one or more new genes may be placed in a chromosome without disturbing its integrity, the number of chromosomes in related species thus remaining the same. On the other hand such additional genes may disturb the existing complex and cause the whole mass of genes to be entirely redistributed, thus causing a change in chromosome number in nearly related species. Should a certain group of genes be placed in one chromosome in one species and in two in another, there would not be necessarily any difference in these two species. It would seem, however,

that there might be a tendency in any large group of related animals for the genes to segregate out according to some definite pattern.

If, therefore, we make a list of all the chromosome numbers which have been reported for all the species of a certain class¹³ of Metazoa, leaving out of account results which are conflicting or are too old to be accurate, we find that a certain number of chromosomes is characteristic of that class; that is, there are considerably more species having that number of chromosomes than any other number. This I will call the 'type number.' The type number of a class of animals is the most frequently occurring number and may be considered tentatively as the fundamental chromosome group. One or more chromosomes of this group or of a group derived from it may split into two (or more) parts, or they may fuse, thus causing the differences in number which occur in related forms. Whether the double groups which occur in closely related forms in many plants (e. g. *Oenothera gigas* and *O. lamarckiana*, *Drossera longifolia* and *D. rotundifolia*, *Spiranthes cernua* and *S. gracilis* etc.) and in some animals (e. g. the bivalens and univalens varieties of *Ascaris megalocephala*, *Helix pomatia*, *Echinus microtuberculatus*, *Artemia salina*; *Cyclops viridis* and *C. gracilis*, *Anopheles* sp? and *Anopheles punctipennis* etc.) are derived in all cases by a splitting of all the chromosomes of the simple group, it is difficult to say. It may be, as suggested by Gates and supported by Strassburger that the double groups are derived in some cases at least, by a failure of cell division after the division of the chromosomes. A slight change in number may also be obtained by the disappearance of a whole chromosome, but this must be rare. All numbers referred to hereafter are the haploid numbers, and X, when present, is counted as one chromosome, even when it consists of several elements.

The type number for the Coelenterates cannot be determined yet, as the data are too scanty and the results conflicting (e. g. *Hydra*). Possibly it is 12. For the Nematelminthes, the type

¹³ The term "class" is used loosely to include related families, orders or classes of ordinary classification.

number is 6, for the Echinoderms 18, for the Amphibia, the only class of Vertebrates satisfactory for generalizations, it is 12. For the Plathelminthes, the type number is 8, for the molluscs 16, for the Annelids 16. As one might expect in a group with so many distinct subgroups, the Arthropods have several type numbers. For the Crustacea it is 8 (the Malacostraca have higher numbers), for the Hemiptera 7, Orthoptera 12, Coleoptera 10, Diptera 6, Lepidoptera 31. It is of interest that the type numbers in the enterocoelous series are all multiples of 6 or 6, whereas those of the teloblastic series (except the tracheates) are multiples of 8 or 8. It is also of interest that the molluscs and Annelids which are so closely related have the same type number 16. The subgroups which are degenerate or highly modified (e. g. Trematodes, Acanthocephala) usually do not have the type number of their groups. The data for the insects are the fullest and the most reliable and these offer the best study of changes in chromosome numbers.

The type number for the Hemiptera is 7 (haploid), including an XY pair or an X. Other numbers occur, all of which can be attributed to the fusion or splitting of chromosomes of the type group. The two *Thyantas* have been a puzzle, owing to their likeness in form and the wide divergence in chromosome number. *Thyanta custator* has a diploid number of 16, which would mean 8 haploid including X or Y. *Thyanta calceata* has 28 in the ♀ diploid, 27 ♂ (owing to X being of two parts, Y of one), which would mean a haploid number of 14 ♀, 13 ♂. These numbers may be explained on the supposition that in *Thyanta custator* one chromosome of the type group has split in two, whereas in *Thyanta calceata* all of these have split except Y. Evidence of this splitting is given by X, which is of two parts, not always separate in *T. calceata*, each about the same size as Y and about half as large as the X in *T. custator*. This explanation may be expressed as follows:

	TYPE	THYANTA CUSTATOR	T. CALCEATA
♀	6+X	6+1+X	6+6+2X
♂	6+Y	6+1+Y	6+6+Y

The same explanation holds for the two *Banasas*, one of which, *B. dimidiata* has a haploid number of 8 (like *Thyanta custator*) and the other, *B. calva* has a haploid number of 13 (like *T. calceata*, except that X is single). *Euschistus crassus* differs from five other species of the same genus which have the type number, in having one less. A comparison of Foot and Strobbe's figures of this species with their figures of the other species would indicate that a union of two large chromosomes has taken place. That the chromosome number does change by the fusion or splitting of chromosomes is shown by the *Notonectidae*. In three species (Browne, '16) there are 13 chromosomes, including two small ones, and in two species there are 12 chromosomes including only one small one. In a sixth species, *N. insulata*, the second small chromosome may be seen attached to another chromosome in the first division of some cells, while in other cells it is free, whereas in the second division it is permanently fused with the other chromosome. The d-chromosome of *Nezara* may also represent a stage in splitting or fusion, as suggested by Wilson. The fact that the X chromosome may consist of two or more parts, as in *Syromastes*, *Phylloxera* and some *Reduvioids*, would indicate that other chromosomes whose identity is not so easily established, may also split into two or more parts.

The type number for the *Diptera* is 6, including XY which are, however, not always distinguishable. There is a decided tendency for the chromosomes to fuse, especially among the *Drosophilas*, most of which have 4 chromosomes, and the *Culicidae*, most of which have 3. In *Anopheles punctipennis*, (Stevens, '11), the X and Y are seen to be attached to another pair in the diploid groups. Metz, '16, has made a careful study of the chromosomes in the genus *Drosophila*, and has shown that many groups having a larger number of chromosomes contain rod shaped ones which are represented in groups with a smaller number by half as many V-shaped ones, two rods uniting to form a V. When the linkage groups in other species of *Drosophila* have been worked out as extensively as in *D. melanogaster*, it may be possible to establish the relation between chromo-

somes of different species and to determine whether fusion of chromosomes has actually taken place.

The Orthoptera have been carefully studied by McClung and his students, and they have found a great constancy, especially among the Acrididae. The type group for the Orthoptera is 12 including X. In *Stenobothrus* (*Chorthippus*), which has 9, Robertson has shown that three of these are really compound. In *Chortophaga*, there is a union of chromosomes in the diploid groups (McClung, '14). In two species of *Hesperotettix* (McClung, '17), X is fused with another chromosome, while in another species, *H. viridis*, it may be fused or free, and fusion may occur among other pairs, correspondingly decreasing the number of chromosomes. Also in *Mermeria bivittata* (McClung, '17) X is fused with another chromosome, while in other species it is free. Among the Locustidae, *Jamaicana* (Woolsey, '15) shows steps in change of number. Some individuals of *J. unicolor* have 31 rod-shaped chromosomes and 2 Vs. in the diploid groups; some individuals of *J. subguttata* have 33 rods and 1 V; other individuals of these two species and all of *J. flava* have all rods and a diploid number of 35. Robertson ('16) has suggested that the 2 V-shaped chromosomes of *Steiroxys* are represented by 4 rods in *Decticus*, giving a total of two more in the diploid group of the latter. An unpublished account of Mohr agrees with this in showing the two Vs in *Steiroxys* and he also shows them in *Locusta viridissima* (diploid number 29) whereas no Vs are present in several other genera whose diploid number is 31. Robertson also points out that several Vs are present in *Gryllus domesticus*, which, if counted as twice as many rods, would give the number of chromosomes in *G. assimilis*; as no figures are given of the latter, this cannot be verified.

There is therefore considerable evidence from the Orthoptera, Diptera and Hemiptera that chromosome numbers change by the splitting and fusion of chromosomes. The splitting of the sex chromosomes, which occurs in many other groups than the insects (see p. 66) indicates the probability that a similar process may take place among the other chromosomes.

Sex chromosomes in insects

SEX CHROMOSOME	GROUP	FAMILY	EXCEPTIONS	TO POLY IN	EXCEPTIONS
X	Orthoptera	Acrididae Blattidae Gryllidae Locustidae Phasmidae	Gryllotalpa; XY Forficulidae?	1st	
X	Hemiptera homoptera	Aphidae Cercopidae Fulgoridae Jassidae Membracidae	Enchenopa bi- nottata (X or XY?)	1st	Enchenopa bi- nottata (X, 2nd; or XY, 1st?)
X	Hemiptera heteroptera	Coreidae Hydrometridae Pyrrhocoridae	Some Metapo- dius (XY)	2nd	Archimerus (1st)
X	Coleoptera	Elateridae Lampyridae One Carabidae Some Chryso- melidae One Silphidae		1st	Photinus, 2 sp. (2nd)
XY	Diptera	Anthomyidae Asilidae Bombyliidae Culicidae Drosophilidae Muscidae Sarcophagidae Statiomyidae Syrphidae		1st	
XY	Hemiptera heteroptera	Belostomidae Galgulidae Lygaeidae Nabidae Nepidae Notonectidae Pentatomidae Reduviidae	Oedancala (X)	2nd	Tingis (Tingiti- dae) (1st)

Sex chromosomes in insects—Continued

SEX CHROMOSOME	GROUP	FAMILY	EXCEPTIONS	TO POLE IN	EXCEPTIONS
XY	Coleoptera	Buprestidae Cerambycidae Cincindellidae Coccinellidae Lucanidae Melandryidae Meloidae Scarabaeidae Staphylinidae Tenebrionidae Some Carabidae Some Chrysomelidae One Silphidae		1st	

V. HETEROCHROMOSOMES

The most conspicuous heterochromosomes are the sex chromosomes, an unpaired X or an unequal XY pair, which occur most characteristically in certain groups of insects. As may be seen from the accompanying table, an unpaired X occurs in practically all the Orthoptera and Hemiptera homoptera and in some families of the Hemiptera heteroptera and Coleoptera; an XY occurs in practically all the Diptera and in some families of the Hemiptera heteroptera and Coleoptera. In some families of the Coleoptera, an X is found in some genera and an XY in others. The sex chromosomes undergo their differential division in the *first* maturation in the Orthoptera, Hemiptera homoptera (except Enchenopa?), Diptera and Coleoptera (except Photinus), and in the *second* maturation division in the Hemiptera heteroptera (except Archimerus and Tingis). Sex chromosomes have not been described in any Hymenoptera, and are not of general occurrence in the Lepidoptera, though here an equal XY in the ♂ has been described in several species, an XY in the ♀ in Phragmatobia and an X in the ♀ in Abraxas. Among the other Arthropods, an X

has been described in some Myriapods, most Arachnids (Araneida) and a few Copepods, but in no other Crustacea and not in Peripatus. Sex chromosomes have not been described in any Annelids, Coelenterates, Nemertines, Porifera, Rotifera, Protochordates or fishes and for only one Plathelminth. A few cases of their occurrence have been reported in the Echinoderms, molluscs, Amphibia, Birds, Reptiles and mammals. In the Nematodes they are of frequent occurrence. In all cases it is the ♂ which is heterozygous except in the Lepidoptera and birds and the genetic evidence agrees with the cytological. Although X and XY are typically single elements, the X consists in some cases of two or more elements closely or loosely associated. An unpaired X of two elements has been described for *Syromastes*, two species of *Phylloxera*, *Leptinotarsa*, *Agalena* (spider), *Hyalocylis* (mollusc), *Schistosomum* (Trematode), *Rhabditis*, fowl, and man and pig; an X of two elements accompanied by a single Y for *Thyanta calceata*, *Grylotalpa borealis*, *Cincindella* and some of the Reduvioids. In other Reduvioids, *Galgalus*, *Notonecta indica* and *Ascaris incurva*, the X of an XY pair consists of three or more parts—of 8 in the last named, and in *Ascaris lumbricoides* and *A. canis*, an unpaired X consists of five and six elements respectively. A Y consisting of several elements has been described for *Phragmatobia*, and a Y of two elements in one testis of *Odontota* (Coleoptera). In a few cases the sex chromosomes are attached to another chromosome during part at least of their history: *Ascaris megalocephala*, *Leptynia*, *Dixippus*, *Hesperotettix*, *Mermeria bivittata*, *Anopheles punctipennis* and *Necturus*.

Another set of heterochromosomes are the supernumerary chromosomes, which typically accompany sex chromosomes and divide in only one division, but are distributed irrespective of X and Y; they are constant in number in an individual, but differ in different individuals of the same species. They have been found in *Metapodius*, *Euschistus variolarius*, *Banasa calva*, *Diabrotica*, *Ceuthophilus*, *Drosophila ornaticornis*, *Circotettix*, *Trimerotropis*, *Hesperotettix viridis*, *Tettigidea parvipennis*, some spiders and *Necturus*.

A third set of heterochromosomes are the m-chromosomes, small chromosomes which remain condensed in the growth period and conjugate late. These are characteristic of the coreid Hemiptera and occur in some of the Lygaeidae; they accompany an unpaired X except in *Metapodius* and *Ichnodemus* where they accompany an XY.

Finally there are those chromosomes which are normal in behavior but consist of unequal parts. To this class belong the d-chromosome of *Nezara hiliaris* and the compound chromosome of *Notonecta insulata*, which divide equally in the two divisions, and the unequal tetrads of some of the Orthoptera which divide into unequal parts in one division: *Schistocerca* (Hartmann, '13); *Acridium granulatus*, *Tettigidea parvipennis* (Robertson, '16); *Arphia*, *Dissosteira*, *Brachystola* (Carothers, '13); and *Phrynotettix* (Wenrich, '16).

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Resumen y traducción por el autor, José F. Nonidez
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Los fenómenos meióticos en la espermatogénesis de Blaps, con especial mención del complejo X.

El número de cromosomas es treinta y cinco, treinta de los cuales se fusionan por parejas durante la sinapsis originando quince bivalentes en el espermatocito primario. Los otros cinco cromosomas se reúnen en un grupo, el complejo X, durante la sinizesis temprana que tiene lugar cuando los cromosomas están aún en el estado de procromosomas. Los cromosomas del complejo X son de tamaño desigual; tres de ellos presentan forma de V y son mucho mayores que los restantes, que no pueden diferenciarse de los cromosomas ordinarios en las espermatogonias. Dos de los grandes cromosomas (denominados M-cromosomas) son homólogos, mientras que el tercero corresponde al cromosoma accesorio. Este último aparece condensado durante el periodo de crecimiento y a él están unidos los M-cromosomas y pequeños cromosomas del complejo. Los cromosomas de este último no se reúnen en parejas durante la sinapsis, sino que permanecen en forma de filamentos independientes durante el periodo de crecimiento. El complejo X se disocia durante la primera mitosis de maduración. Uno de los M-cromosomas entra en uno de los espermatocitos secundarios, que recibe en total diez y seis cromosomas. El otro espermatocito recibe diez y nueve cromosomas, incluyendo los cuatro elementos restantes del complejo. El complejo X parece representar una condición intermedia entre los complejos formados exclusivamente por cromosomas sexuales y los originados por la asociación de un cromosoma sexual con un par de cromosomas ordinarios.

THE MEIOTIC PHENOMENA IN THE SPERMATOGENESIS OF BLAPS, WITH SPECIAL REFERENCE TO THE X-COMPLEX

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EIGHTY-SIX FIGURES

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I. INTRODUCTION

The formation of the male germ cells of *Blaps lusitanica*, a large beetle belonging to the family Tenebrionidae, has engaged my attention for more than three years, in an attempt to trace the history of the chromosomes throughout the spermatogenesis. In a first paper ('14) I described in detail the spermatogenesis and the behavior of a peculiar complex—the X-complex, made up of three large chromosomes—throughout the postsynaptic stages and first maturation mitosis. Further progress was made later ('15) and the results obtained in this form confirmed in *Blaps waltli* Seidl., a closely related species which possesses a similar

complex. In both papers, however, conflicting results were reached with reference to the number of chromosomes and the composition of the X-complex in *B. lusitanica*, which seemed to be variable even in the same individual. It was soon realized that these conflicting results were due to imperfect observation rather than to differences in the process of reduction and that further study was needed in order to solve the question.

The present paper is the result of a new investigation of the whole subject, including the reduction of the chromosomes and especially the origin and behavior of the X-complex. The re-

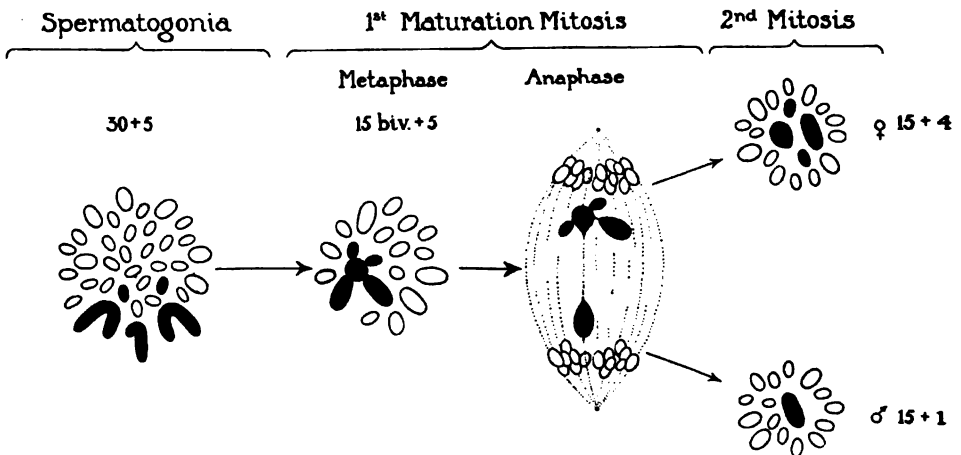


Diagram 1

sults described in the following pages agree, in the main, with those recorded in my previous papers, so far as the features of reduction are concerned, but the conflicting results on the composition of the complex have been cleared up and the conditions upon which the doubts were based carefully compared. I have been fortunate in obtaining a series in which the true composition of the complex could no longer be doubted, since its components are most clearly shown.

Briefly stated, in the spermatogonial mitoses of *B. lusitanica* thirty-five chromosomes occur, three of which are remarkably large (diagram 1). During synapsis the three large chromosomes and two of the smaller (represented in black in the diagram)

unite to form the X-complex, while the other chromosomes pair to form fifteen bivalents. In the first maturation mitosis the complex undergoes dissociation, four chromosomes—two large and the two small—passing to one cell, while the third large chromosome enters the other. The final result is the production of two kinds of spermatozoa, one with nineteen chromosomes, the other with only sixteen.

A remarkable feature of the meiotic phase in *Blaps* is that the complex can be recognized in the spermatocytes at every stage, from the early synapsis to the metaphase of the first maturation division, owing to the fact that one of the large chromosomes appears condensed, like the so-called accessory or X-chromosome of other animals, while the other two large chromosomes persist in the form of long threads united with it. Furthermore, it has been possible to find that the latter do not pair at synapsis, but remain as separate individuals, a fact which led me ('14) to the conclusion that telosynapsis takes place in this case, although the actual pairing of the other chromosomes was never observed.

There are still several points which are left unsolved, since the material in my possession is not especially fit for their solution, but a comparative study of the conditions in other species of *Blaps* and the preparation of new material of *B. lusitanica* might give the clue to the phenomena described in the following pages.

It gives me great pleasure to express my deep indebtedness to Prof. Edmund B. Wilson, to whom I owe sympathetic interest and valuable criticism throughout my work; also to Prof. Frank R. Lillie, Director of the Marine Biological Laboratory of Woods Hole, for the kind hospitality extended to me in that institution during the summer of 1918. Finally, and not the least, to the Junta para Ampliación de Estudios e Investigaciones Científicas of Madrid, for the fellowship granted, through which it has been possible to continue my studies in this country.

II. MATERIAL AND METHODS

Blaps lusitanica Herbst is common in woods and uncultivated places near Madrid, Spain, and is usually found in the holes inhabited by rabbits, which it does not leave except at sunset and during the night. All the individuals studied were collected almost in the same spot, in a place known as Puerta de Hierro, during three consecutive years. The specimens were killed by means of chloroform vapor shortly after their capture. Several preserving fluids have been used with variable results; Bouin's fluid, Lenhossek's alcoholic sublimate, and Carazzi's modification of Gilson's fluid proved to be the best, and the most valuable slides were thus obtained. Zenker's fluid and Vom Rath's picro-acetic sublimate gave rather good results, but the mitochondria appear stained to some extent when using iron hematoxylin, and this hampers the accurate study of the chromosomes in the maturation divisions; for this reason these fluids were not used extensively. Flemming's strong and weak solutions have been a complete failure in my hands, the testes being so large that the reagent penetrates with difficulty, while the thick adipose tissue enveloping the organ reduces the osmic acid and prevents its access to the interior of the follicles; hence only the periphery of these is suited for study.

The testes embedded in paraffin were cut into sections from 6 to 10 μ thick. A few stains have been used, most of the slides being stained with iron hematoxylin, orange G and light green as counterstains. The results obtained with safranin and light green, after preservation with sublimate mixtures or Bouin's fluid, proved to be very meager.

I have never succeeded in making smears and hence have been deprived in my study of the valuable results thus to be obtained.

III. THE SPERMATOGONIAL MITOSES

Seriation of the stages. A very important and often difficult point in the study of spermatogenesis is the seriation of the stages. In *Blaps* the seriation is very easy on account of the way in which the cysts are produced. Although the latter arise

in the terminal cap, which is a zone occupying the distal end of each follicle, very often only those produced in its central area are apt to go through all the stages of spermatogenesis; while those lying in the periphery usually degenerate. Thus a stream of cysts, as it were, containing all the stages, is produced. Furthermore, the cells of a cyst usually do not appear in the same stage; this is specially true in the case of those dividing. One can find, for instance, even in the same section, all the stages connecting the metaphase with the late anaphase. This happens not only in the spermatogonia, but also in the spermatocytes, and owing to this fact more than one point which otherwise would appear puzzling has been settled beyond any doubt.

The spermatogonial mitoses. Since in my first paper ('14) I dealt extensively with the spermatogonial mitoses, it seems unnecessary to give a detailed account of them now. The main stages have been represented in figures 8 to 35, which speak for themselves. Only a few details are considered here, most of them outlined in my previous work.

During the resting stage of the early generations of spermatogonia, the chromatin is scattered throughout the nuclear cavity, forming irregular masses made up of granules, located near or at the periphery, in contact with the nuclear membrane. A large chromosome occurs in these cells, which retains more or less clearly its individuality throughout the resting condition; its appearance varies greatly according to the size of the chromatin masses of which it is made up, which may be very small and regular, placed along a thread (figs. 1, 2, and 7, *a*), or coarse and unequal (figs. 3 to 5 and 7, *b*, *c*). In some cases it appears as a condensed, more or less V-shaped chromosome of ragged outline (fig. 6). Whether or not this body represents the X-chromosome which later appears condensed in the spermatocyte is a question rather difficult to answer. However, the fact that it retains its individuality and that in the early spermatocytes a similar chromosome is found, which could be traced to the condensed body of the later stages, makes this assumption highly probable.

In all the individuals studied the spermatogonial metaphases contained normally thirty-five chromosomes (figs. 16 to 20). Three of them are readily identified on account of their large size and peculiar shape, as they almost always appear as curved, V-shaped rods, rarely straight or slightly bent, the arms of the V being of unequal length. Although their position in the metaphase is variable it is commonly peripheral.

It has been possible to find a correspondence in shape and size between two of the large chromosomes (figs. 16 to 20, *M*, *M'*), while the third chromosome differs from them in both shape and size. The latter was identified in my former papers as the accessory or X-chromosome. The paired large chromosomes were first termed 'chromosomes *a* and *b*' ('14, p. 36) and subsequently 'macrochromosomes' or more simply, 'M-chromosomes' ('15, p. 150), which name I will use in the present paper.

In spite of some variation in shape and size in the large chromosomes, found even in the same individual (fig. 21), the differences mentioned above are almost always present. Furthermore, their mode of attachment to the spindle fibers, better seen in the early anaphase (figs. 23 to 27), strengthens this view. While the attachment is telomitic (Carothers, '17) for all the small chromosomes (fig. 27), it is clearly atelomitic for the large ones, but a difference is perceptible in the latter: the M-chromosomes possess a submedian attachment, while that of the X-chromosome is subterminal or nearly so. These conditions are already detected in the equatorial plate, as shown by the bending of the rod, except when the chromosomes appear as straight or slightly curved rods (fig. 21 *a*, *c*, *d*, *e*), in which case it is very difficult to ascertain the point of attachment of the fiber. No telomitic attachment in these chromosomes was ever found; it seems quite possible that the bending may appear when the spindle fibers begin to pull the two halves apart.

The small chromosomes appear as oval or elliptical bodies which have no constant position in the metaphase. In normal cells their number is thirty-two, but occasionally one or two more, or fewer may appear. In the first case this is probably due to their fragmentation; in the second to clumping. On the

whole, their number is fairly constant in every individual studied. I have failed in several attempts to arrange the small chromosomes in pairs. In a few cases this could be done, but their small size prevents accurate measurements and comparisons. It is obvious that at least two or three pairs can be recognized, but it is very difficult to find such a pairing in the others.

The two small chromosomes which enter the complex are not distinguishable from the others either in shape or size. Since the other thirty pair during synapsis, giving rise to fifteen bivalents, it seems advisable to speak of them as the euchromosomes (McClung, '14) in the description of the spermatocytes.

During the resting condition of the spermatogonia the centrosome is placed somewhere between the nucleus and the mitosome or spindle remnant; this is specially seen when the cells assume a conical shape, the mitosome in this case being placed at the pointed end. Later on, during the prophase, the centrosome comes in contact with the nuclear membrane—if it was not before—and the astral rays are conspicuous at this time (fig. 13).

The centrosome takes this position during the telophase or even at the beginning of this stage (fig. 31), moving along one of the sides of the nucleus. Figures 32 to 34 show some stages of this migration. In figure 32, upper cell, the centrosome, which since the late anaphase has two minute centrioles, still keeps its position in one of the poles of the already vanished spindle, but this position is unusual. In the lower cell of the same figure it is seen at the left of the nucleus. In figure 33 it is clearly detected in the lower cell, while in figure 34 it appears in both daughter cells. When the remnants of the spindle contract and condense to form the mitosome, the centrosome takes its final position (fig. 35) and upon elongation of the cell body usually loses all contact with the nuclear membrane.

As a further proof supporting this account, it must be stated that in some spermatogonia in which the oval shape of the telophasic nucleus is retained, the free ends of the large chromosomes, as seen in the prophase, are directed toward the centrosome (fig. 12); had not the latter moved around the nucleus, the conditions would be the reverse, with the apices of the loops nearer

the centrosome. This might be accomplished by a rotation of the nuclear contents, as described in the early spermatocytes of *Batrachoseps* by Janssens ('03, '05). Such, however, is not the case in *Blaps*; since the mitosome does not move from its place of origin and often may be recognized more or less clearly during several cell generations, it offers a fixed point of reference. If a rotation of the nuclear contents took place, the centrosome would always appear in the half of the cell opposite to that containing the mitosome. Furthermore, no telokinetic movements of the nucleus were observed, as the figures show; even if the nucleus turned around the centrosome (the latter retaining its primitive position), the apices of the loops would still be directed toward the centrosome.

IV. THE SPERMATOCYTES

1. *The maturation mitoses*

The first maturation mitosis is reductional for the X-complex. In the course of the two maturation divisions the bivalent euchromosomes, as usual, divide along two planes cutting each other at an angle of 90° and their transverse fission takes place in the first mitosis. It seems likely that the latter segregates two homologous chromosomes, paired during synapsis and placed end to end in the metaphase, but we lack conclusive proof of this conclusion. The absence of conspicuous tetrads makes very uncertain the recognition of the spacial relation of the chromatids in each pair, and this is more important than the direction of the plane along which division takes place. The fact that the X-complex undergoes dissociation during the first mitosis does not prove the reductional character of this division for the euchromosomes, since several cases have been described in which the behavior of the sex chromosomes differs in this respect.

A. *First maturation mitosis. Prophase.* At the end of the prophase, when the condensation of the chromosomes makes possible their certain recognition as separate individuals, these bodies may be grouped in two sets, one represented by the large X-complex (fig. 36, b), the other by fifteen bivalent euchromo-

somes, the total number being sixteen, counting the multiple complex as one (fig. 37).

The appearance of the chromosomes in the early prophase is somewhat different. Unfortunately, it was not possible to trace in detail all the changes the euchromosomes undergo and the manner in which they are brought about, because of their small size and to some extent also because of the methods of preservation used. The evidence on this point is so meager that I will offer only a short description of this interesting process, without any attempt to draw conclusions of general bearing.

At the end of the growth period there is a diplotene stage during which the euchromosomes appear longitudinally double, each half probably representing an univalent chromosome. The diplotene stage is of a short duration, passing directly into the condensed chromosomes found in later stages without an intervening confused stage such as occurs in other beetles. In some of the euchromosomes the splitting is not complete, both halves being connected with each other at one of their ends, and in this way V's or Y's are formed (figs. 39 and 40, *a, e*). The splitting may also begin at both ends of the chromosome, the halves thus produced being united at the center (fig. 40, *f*) often by several anastomoses. When they diverge at the ends X's arise, although it is quite possible that in this case both halves are crossed over each other (fig. 40, *g*).

These facts point to the existence of different methods leading to the formation of the condensed bivalents and, probably, these methods are related to individual chromosomes, but no conclusive evidence on this point has been obtained so far.

No longitudinal cleft was detected in the halves of the bivalents at any stage of condensation. I do not know whether this is a special feature of this species or the result of preservation. No conspicuous tetrads occur, therefore, and the rings and crosses so characteristic of other insects were never observed.

Once these conditions are reached, a condensation of the euchromosomes takes place to form the oval or slightly dumb-bell-shaped bodies found at the end of the prophase (figs. 36 and 37). Different steps leading to these conditions appear within the

same nucleus, as shown in figure 38, in which all the chromosomes of a cell were drawn in their actual position, while in figure 39 they were arranged in a series. Euchromosomes 1 to 3 show a more or less conspicuous splitting, while in the one marked 4 the two halves diverge to produce a V. Euchromosomes 5 and 6 appear as Y-shaped bodies, seen endwise in 7, 8, and 9. The other chromosomes are well on the way to condensation, which seems to be combined with a displacement of the halves of each bivalent in such a way that both components, which at first were placed side by side, come to lie end to end.

The X-complex appears at the end of the prophase as an approximately V-shaped body (figs. 36, *b*, and 37). Its apex is occupied by a round or oval chromosome which owing to its dense condition retains the stain longer than any of the other

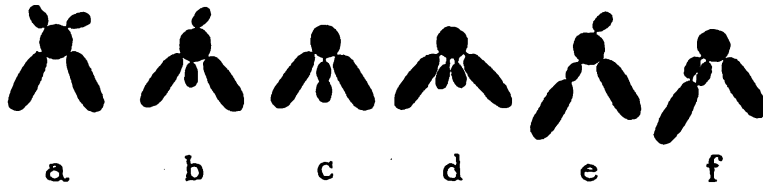


Diagram 2

chromosomes, even in much decolorized slides. This body, as shown by its history throughout the growth period, is the accessory or X-chromosome.

The arms of the V-shaped complex are formed by the M-chromosomes which are less dense. In some complexes the X-chromosome is located in the free end of one of the latter instead of being at the apex of the V, a condition found also in the metaphase.

The small chromosomes of the complex are united with the X-chromosome, but their position is highly variable as shown in figure 41 and diagram 2. In a few cases one may be united with one of the M-chromosomes, as seen in the same figure *d*, which shows one condensed and lying over the X-chromosome, while the other is still a thin thread connected with one of the M-chromosomes. In most cells, however, the first condition pre-

vails. At the end of the prophase they are as condensed as the euchromosomes.

In many complexes only one small chromosome is seen (figs. 38, 39, and 41, *e*), if they are seen at all, the number of euchromosomes in the cell being the normal. Their presence in the complex is, however, evident since the number of the euchromosomes is not increased, a fact still clearer in the metaphase. But an even more conclusive proof is their appearance in the early anaphase, which shows that, at least in some cases, they are placed between the X and one of the M-chromosomes, which fact may also account for the unequal length of the arms of the V-shaped complex, very conspicuous in some cases.

In sharp contrast with the conditions described in the euchromosomes, the components of the X-complex never appear split lengthwise, either during the short diplotene stage or in subsequent stages of the prophase. In figure 30 of my first paper ('14) I have represented one of the M-chromosomes as split, but this must have been an abnormal condition since I have not been able to find a similar cleft in other cases.

The only change undergone by the complex consists of a condensation of its components (with the exception of the already condensed X-chromosome); this process takes place in several regions (chromomeres) along the chromosomes, hence their segmented appearance. Very often the V-shape of the complex is not assumed until the late prophase, the M-chromosomes appearing in early stages united by their free ends, almost parallel in some instances, curved as to form a ring in others, and in not a few cases crossed over each other (fig. 41, *a*, *e*).

Metaphase. Figures 42 to 47 show polar views of metaphases drawn from different individuals, with the exception of figure 46, which shows four optical sections of a metaphase seen in lateral view. Inspection of such figures shows the irregular position of the euchromosomes with regard to the X-complex, which may occupy the center of the plate or may be located at the periphery; very often it is placed at a different level. Although its presence in normal spermatocytes is absolutely invariable, its shape is highly inconstant. In favorable cases all five components can

be detected (figs. 42 to 44, 46 to 48, *a*, *b*), but in many metaphases only one of the small chromosomes is seen (figs. 45 and 48, *c*), and in others both seem to be absent.

An unusual form of complex is represented in figures 47 and 48, *b*, in which the X-chromosome failed to condense at the beginning of the growth period and appears as a curved, more or less V-shaped body, united at the ends with the M-chromosomes. The small chromosomes are easily recognized here, lying at slightly different levels and connected with the X-chromosome by means of delicate threads.

Although the preserving fluids used are not the best to show clearly the spindle fibers, it was possible to detect their mode of attachment to the complex. The normal attachment is seen in figure 46, *b*. One spindle fiber is attached to the X-chromosome, another to one of the M-chromosomes; the other M-chromosome and the small chromosomes lack a spindle fiber of their own. There are, however, some variations in this respect, which will be described later. The euchromosomes possess a double attachment, both fibers being attached at their ends so that their longer axis is parallel to the axis of the mitotic figure.

Anaphase. The most interesting feature of the first maturation mitosis is the distribution of the chromosomes involved in the X-complex among the secondary spermatocytes. As shown in diagram 1, one of the latter receives four of such chromosomes, while the other receives only one. Furthermore, on account of the almost constant position of the X-chromosome in the complex, it was possible to detect its entrance into the spermatocyte receiving four chromosomes. In this way two kinds of secondary spermatocytes arise, one in which the total number of chromosomes is nineteen—15 euchromosomes plus 4 X-complex components—the other with sixteen chromosomes—15 euchromosomes plus 1 X-complex component.

This distribution is constant in normal cells, but in a few cases the large chromosomes enter the same spermatocyte; there is, however, every reason to believe that the latter does not produce a functional spermatozoon. The irregularities in the distribution of the small chromosomes are of a somewhat wider occur-

rence, judging from the slight numerical variation found in the secondary spermatocytes.

The dissociation of the complex usually takes place when the already divided euchromosomes are on their way to the poles of the spindle. It starts with the loosening of the links which hold the chromosomes together, followed by their partial separation; for, in the early anaphase at least, they remain bound together by means of connecting fibers (figs. 49 to 51). The X-chromosome and that M-chromosome which possesses a fiber attachment separate and pass to opposite poles of the spindle, while the remaining M-chromosome (which lacks a spindle fiber) accompanies the X-chromosome (figs. 49 to 52). In the late anaphase, when the euchromosomes become crowded at the poles of the spindle, it is still possible to detect the large chromosomes outside of the clumped smaller ones and thus to be sure of their final distribution.

The two small chromosomes of the complex are easily detected when only fused with the X-chromosome, as they appear as more or less conspicuous knobs on the surface of the latter (figs. 49 and 50); in other cases, however, their recognition is not possible until the components of the complex have entirely separated. I was at first inclined to admit their absence in such complexes, but, fortunately, the presence of a closely graded series of stages in a single cyst enabled me to trace their history during the anaphase, which has been confirmed by the inspection of many other cells in mitosis.

The study of the complexes undergoing dissociation shows that the small chromosomes may be situated between the X-chromosome and the M-chromosomes, and hence they escape detection in earlier stages. When lying between the X-chromosome and the antagonistic M-chromosome (which passes to the opposite pole of the spindle) at the beginning of dissociation, a connecting bridge appears between the X-chromosome and the M-chromosome (fig. 54, *a*); in some cases this bridge is split lengthwise (*b*). As the separation of the large chromosomes progresses the small ones gradually emerge from this connecting tract and finally become conspicuous, lying either end to end (*e*) or side by side

(c, d). The split condition of the bridge seems to be connected with the last position.

It is obvious that in the normal anaphases both small chromosomes go into the same cell as the X-chromosome, as proved by the number of chromosomes present in polar (fig. 53) and lateral views (figs. 49 and 50) of the late anaphase. In the latter they are well separated from the large chromosomes but always located in their immediate vicinity, the whole group lagging behind the euchromosomes (figs. 51 and 52). On the other hand, conditions similar to that represented in figure 54, *f*, point to irregularities in their distribution; the small chromosomes of this complex appear to pass to the cell which does not receive the accessory. It is questionable whether both enter the same cell or whether they separate a little later, but in any case the close connection of one of them with the M-chromosome leads to this conclusion. This unusual distribution might be explained as a result of the passive rôle of the small chromosomes and their connections with the other components of the complex.

One of the causes of the abnormal distribution of the chromosomes of the complex is found in the fact that the spindle fibers may have an attachment different from that described in the metaphase. In a few instances neither M-chromosome is attached to a spindle fiber of its own; in this case the attachment is normal for the X-chromosome, but one of the small components has taken the place of the former. The result in this case may be inferred upon inspection of figure 55, *b, c*. The small chromosome, which here takes the place of one of the large ones, probably does not separate from the others, but enters the same cell with them, although it is considerably stretched. The normal distribution of the chromosomes is, however, not brought about, for both M-chromosomes now behave as passive bodies and are drawn into the same cell. This may account for the existence of secondary spermatocytes containing all the components of the complex together with the haploid number of euchromosomes, a condition which at first appeared very difficult to explain, for had the separation of the daughter cells failed to take place, the diploid number of euchromosomes would have been retained.

In other cases the distribution of the euchromosomes is quite irregular, the number of chromosomes in the secondary spermatocytes showing a surprising variation. I wish merely to emphasize this fact without attempting to give its explanation, since positive facts concerning the irregularities of distribution are lacking at present.

B. The secondary spermatocytes and the second maturation mitosis. At the end of the anaphase of the first mitosis the chromosomes become crowded in the poles of the spindle. When the daughter cells separate they become looser and are disposed near the periphery of a vacuole-like nucleus, without losing their dense appearance, although they become a little ragged in outline. By this time the spindle of the second division has appeared and soon all the chromosomes enter in the metaphase.

In the latter two large chromosomes of the X-complex, slightly elongated and quite separated from each other, can be recognized in about half of the cells, while the other half has only one such chromosome (figs. 56 and 58). The small chromosomes are no longer distinguishable from the euchromosomes.

The numerical variation already referred to is very conspicuous at this time. Figure 59 shows a metaphase of a spermatocyte lacking the X-chromosome in which seventeen chromosomes occur. This number may be explained on the assumption that both small chromosomes passed to the cell without the X-chromosome, as suggested by figure 54, *f*.

Corresponding variations occur in spermatocytes with the X-chromosome, where the normal number may be decreased by one or two (fig. 57). There is always the possibility in this case that one or both small chromosomes may be still fused with the X-chromosome and do not appear as separate individuals, but the increase in the number of chromosomes in the spermatocytes of the other kind clearly shows that irregularities certainly occur.

The division of the chromosomes takes place in the same way as in the spermatogonial mitoses, namely, the chromosomes split lengthwise and the halves thus produced are distributed among the spermatids (figs. 60 to 63). The latter no longer show condensed chromosomes and all appear alike despite the differences

of the chromatin contents due to the unequal distribution of the chromosomes of the X-complex during the reductional mitosis.

2. The growth period

The absence of a confused or reticular stage in the growing spermatocytes makes possible the recognition of independent chromosomes throughout the greater part of the growth period. No attempt has been made to trace in detail the history of the euchromosomes, but their condition in the different stages is described for comparison with that of the chromosomes of the X-complex. The behavior of the latter is extremely interesting and stands in sharp contrast with that of the euchromosomes, since they are brought together slightly before synapsis, but remain independent to some extent forming a loose complex, without being actually paired and, therefore, show their univalent nature throughout the postsynaptic stages.

The presynaptic stages can be classified as follows:

a. The resting stage, which is but an extended telophase of the last spermatogonial mitosis, the nuclei closely resembling in structure those of the spermatogonia.

b. The prochromosome stage, during which the chromosomes appear as massive bodies located near the periphery of the nucleus.

c. The synizesis stage, in which the prochromosomes move to that pole of the nucleus which is nearest the sphere and centrosome, as in the ordinary contraction figure.

d. The unraveling stage, representing the transition between the prochromosomes and the leptotene threads, the chromosomes appearing as convoluted threads.

e. The leptotene stage, in which thin threads occur.

Stages *a* and *c* are highly characteristic of Blaps. The prochromosomes have been described several times in animals, but in no case, I believe, has synizesis been described when the chromosomes are in such a condition.

Synapsis probably takes place when the leptotene stage is reached, but on account of the crowded condition of the chromo-

somes, which still occupy one of the halves of the nucleus, it is very difficult to observe their actual pairing. Moreover, since the X-complex arises before the leptotene threads are formed, it is possible that synapsis takes place also in the prochromosome stage. Therefore, it seemed inadvisable to recognize a definite synaptic stage.

The postsynaptic stages are the following:

f. The early pachytene stage, in which the bivalent euchromosomes are confined to one of the nuclear hemispheres and produce a condition which could be compared to the bouquet stage in other animals.

g. The pachytene stage proper, with thick and apparently undivided threads scattered within the nucleus.

h. The diplotene stage, during which the euchromosomes split lengthwise; this stage leads gradually to the prophase of the first maturation division.

Since the behavior of the chromosomes of the X-complex differs from that of the euchromosomes, it has seemed advisable, for the sake of clearness, to consider it separately.

A. Presynaptic stages and synapsis. a. Resting stage. There is no doubt that a resting stage intervenes between the last spermatogonial anaphase and the prochromosome stage, for, besides the evidence furnished by the seriation of the stages, spermatocytes in both conditions were found within the same cyst (fig. 64).

With exception of the spermatids, the resting spermatocytes are the smallest cells in the testis. The chromatin appears in their nuclei as scattered masses very similar to those described in the spermatogonia (fig. 65); as in the latter, a deeply stained mass of chromatin can be detected sometimes, resembling more or less closely a chromosome with very ragged outline, often broken up into several masses. This body is the X-chromosome and its history could be traced to the succeeding stages.

The condition of the chromosomes is brought about by the same process as in the spermatogonial anaphases. In no case do the chromosomes retain their dense condition, but they pass into the stage just described, which appears to be of rather long

duration, if we may judge from the considerable number of cysts showing such cells.

b. Prochromosome stage. In this stage (figs. 67 to 70) the chromatin appears in the form of condensed bodies located at the periphery of the nucleus in close contact with the nuclear membrane; hence in optical section the center of the nucleus is deprived of chromatin (fig. 67, cell to the right). The number of massive bodies, which I formerly called 'cromosomas preleptoténicos' (since from each of them a leptotene thread arises), agrees with the diploid number, as already stated in my second paper ('15, p. 157). Reexamination has proved the correctness of this assumption. In some cells it is very difficult to be sure of their exact number, but even so we can be almost certain that it seldom exceeds or falls short of the diploid number. Thus, in the cells represented in figure 70, in which the prochromosomes were drawn from the same cell, thirty-six dense bodies occur. This slight increase may be due either to one of the less dense masses being nothing but residual chromatin or to one chromosome being cut into two, as drawings *c* and *d* are from different sections. The large chromosomes of the complex can be distinguished in most of the cells, a description of them being given later.

The process by which the prochromosomes arise is worthy of mention. The scattered chromatin granules gather in certain places (fig. 66) and, upon further condensation, form the prochromosomes. Although these resemble very much the chromosomes of the metaphase in the spermatogonial mitoses, no threads take part in their formation; hence we cannot speak of a true prophase, since the difference between the two processes is obvious. I would suggest that this process might well be called a 'pseudo-prophase,' since the dense chromosomes arise from chromatin masses without passing through the normal stages of spireme chromosomes. An early stage of this process is represented in figure 66. The cell to the left in figure 67, which represents a polar view of a spermatocyte, shows prochromosomes of irregular outline which precede the denser bodies. The condensation, however, never goes so far as to produce the chromosomes with smooth outlines characteristic of mitosis.

c. *Synizesis*. So far as the euchromosomes are concerned, very little can be added to my former account of this stage ('14, p. 55). Once formed, these bodies begin to shift their peripheral position and become crowded near that pole of the nucleus nearest the sphere and centrosome. The degree of contraction is slightly variable according to the individual; in some specimens a mass of clumped chromosomes is formed, in which it is impossible to detect individual chromosomes, while in others the process does not go so far, although the chromosomes become restricted to a half of the nucleus.

This stage represents the synizesis of other forms, with only the difference that, whereas in the latter the contraction usually takes place after the leptotene threads have arisen, in Blaps it occurs at an earlier stage. Figure 71 shows the beginning of the process. In figure 72 two cells in this stage have been represented; the nucleus in the cell to the left is seen in optical section. Figure 73 shows the same condition as seen in polar view.

d. *Unraveling stage*. This stage is marked, as in other forms, by a resolution of all the prochromosomes (with the exception of the X-chromosome) into closely convoluted threads which then unravel to form the thin threads of the succeeding stage. This process is rather difficult to follow in the euchromosomes on account of their small size and crowded condition at the pole of the nucleus, but in the M-chromosomes it is almost as conspicuous as in the best objects that have been studied (fig. 76, a, b).

The polar position of the prochromosomes is retained during the unraveling stage. Figure 75 shows some phases of this process in a spermatocyte seen in polar view; some threads have already appeared, other prochromosomes are unraveling and a few are still in the massive condition.

e. *Leptotene stage*. The leptotene threads produced by the process just described are restricted to the pole of the nucleus in contact with the sphere. In polar views it is possible to detect the presence of delicate threads, somewhat wavy in appearance, the shortest of which are the euchromosomes and small components of the X-complex (fig. 77). Two other threads, much longer, conspicuous, and connected with a dense mass, are the

M-chromosomes, which also undergo a process of unraveling. In side views of the nucleus all that can be seen is a mass of closely interwoven threads from which some filaments emerge, spreading in the nuclear cavity (fig. 78, left of the nucleus), but on the whole they remain crowded at the pole nearest to the sphere. A comparison between the euchromosomes and M-chromosomes fails to show any other difference than the greater length of the latter.

Synapsis. Observation of the actual pairing of the euchromosomes to form the pachytene threads is exceedingly difficult in *Blaps* on account of the contraction of the chromatin and, in spite of several attempts to solve this question, no positive results could be obtained. It is true that here and there it is possible to find parallel leptotene threads which might be regarded as the early zygotene condition, but the Y figures, so conspicuous in the nuclei of the spermatocytes of other animals, and the progressive formation of thick threads by the close association of a pair of leptotene threads could not be detected in my material.

The question of synapsis is therefore unsettled, so far as the euchromosomes is concerned. Judging from the clefts occasionally seen in the euchromosomes during the early pachytene stage and the longitudinal split seen before the prophase of the first maturation division, some ground is given for the belief that parasynapsis takes place.

B. Postsynaptic stages. f. Early pachytene stage. Nuclei in this stage are found in cysts containing spermatocytes in the leptotene condition. The stage is characterized by the presence of thick rods, straight, slightly curved, or very often V-shaped. In a few of these rods (fig. 80) a longitudinal cleft or space may be detected, which seems to correspond to a place in which the conjugating chromosomes are still separated. In slightly later stages this cleft disappears, the euchromosomes becoming thick and continuous throughout (fig. 79).

As a result of the pairing of the chromosomes, the dense condition of the synaptic knot gives way to a somewhat looser mass which begins to spread slowly throughout the nuclear cavity. There is no definite orientation of the chromosomes at this stage.

A few V-shaped rods have their free ends directed toward the sphere, but this is inconstant. It is questionable whether their arrangement is merely accidental or constant for some euchromosomes, the former assumption being most probable. Hence it is not possible to speak of a bouquet stage. This term was adopted in my first paper, but the comparison with forms in which this stage appears very marked shows that a great difference exists in this respect.

g. Pachytene stage proper. The chromosomes in this stage appear spread throughout the nucleus without definite orientation and are probably connected with each other by means of very delicate threads. As in the preceding stage, each euchromosome is a thick rod in which, at first, no split can be detected. In the late pachytene nuclei, however, a few chromosomes show the beginning of the longitudinal split of the succeeding stage. The bivalent euchromosomes are continuous throughout; the chromatin granules do not show an arrangement into two series, which would suggest the existence of two conjugated chromosomes.

h. Diplotene stage. As already mentioned, this stage lasts but a short time and immediately precedes the formation of the dense bivalents of the first maturation mitosis. It is questionable, therefore, whether we should consider such a condition as a separate stage. Nothing can be added to the description already given in the preceding pages.

C. The X-complex during the growth period. The X-complex is formed at the beginning of synizesis. Its components, separated up to this time and often scattered throughout the nucleus, come closer together and finally form a group which, from this moment, behaves as a single unit, moving to the pole nearest to the sphere along with the euchromosomes (fig. 71). At least the three large components can be detected and, in favorable cases, one or two smaller masses, which represent the small chromosomes, are also found in close contact with the former. One of the large chromosomes appears somewhat denser than the others and retains this condition in the succeeding stages. This body is the X- or accessory chromosome. Its earlier history differs

from that of the M-chromosomes. When the prochromosomes are condensing the X-chromosome appears as a V- or J-shaped body (fig. 67, cell to the left); once the massive condition of the former is reached, it becomes looser in structure, finally unraveling in a thick, coarse thread of ragged outline (figs. 68, 69, and 70, *a*). A condensation follows and the deeply stained body of later stages is formed. The process of condensation must take place rapidly, since I have not been able to find stages leading to this condition. Even when found it would be very difficult to decide whether condensation or unraveling is taking place.

It may happen that one of the M-chromosomes also unravels at this time, or at least becomes looser in structure (fig. 70, *M*); perhaps this is but the result of deferred condensation. The close proximity of this M-chromosome and the X-chromosome in the figure and their connection by means of delicate fibers is suggestive of an influence of the latter on the M-chromosome, but conclusive evidence on this point is lacking, since this appearance is far from common.

The M-chromosomes, and probably also the small chromosomes, behave from this moment like the euchromosomes, so far as the unraveling process is concerned. When the latter begin to unravel, the M-chromosomes become looser in structure, and each gives rise to a tightly convoluted thread which, on account of its considerable length, spreads throughout the nuclear cavity. Two details of the unraveling process are shown in figure 76; in figure 78 the leptotene M-chromosomes still exhibit a wavy appearance which is less conspicuous in those of figure 77. It is very difficult to follow the unraveling of the small chromosomes, since in most of the cases the X-chromosome, to which they are united, lies in the mass of chromosomes located in one of the nuclear poles.

It is an interesting fact that the M-chromosomes preserve their original thickness during the early pachytene condition and appear as separate individuals connected with the X-chromosome by one of their ends. A comparison of figures 77 and 78 (which represent the leptotene stage) with figures 79 and 80, which are nuclei in the early pachytene stage, shows this condition very

clearly. In figure 80 a portion of the M-chromosomes has been cut by the knife, the X-chromosome appearing separated from the former. These relations are preserved in figure 79.

A little later the M-chromosomes become shorter, and correspondingly thicker. Assuming that parasynapsis occurs in these chromosomes, we ought to find some cells in which a single thick thread is connected with the X-chromosome, but careful search failed to reveal such a condition, the relations just described occurring in hundreds of cells. Occasionally the M-chromosomes are not visible, being entangled in the mass of euchromosomes, but wherever they appear one can be sure that they are separated. During the pachytene stage proper the complex stands out quite sharply within the nucleus. It is possible to detect the relations of the M-chromosomes and the accessory in almost every cell, and in many complexes the small chromosomes are united with the accessory. Perhaps the clearest case of this is represented in figure 26 of my first paper, in which this relation was thought to be accidental.

At this time two varieties of complexes are found. In one the X-chromosome is situated between the two M-chromosomes (figs. 82, 83, and 84) which are often united by the opposite ends by a very thin thread. In the other kind, already represented in the paper just mentioned, the X-chromosome is at the extremity of one of the M-chromosomes, which are directly united with each other. I do not believe that the last arrangement is accidental, for similar complexes occur rather often during this stage as well as during the prophase and metaphase of the first maturation division. That the M-chromosomes are united directly by one of their ends is obvious, for the length of the single thread thus formed is approximately twice that of each M-chromosome (figs. 81, 85, and 86).

It is interesting to note that the length of the M-chromosomes is sometimes unequal. Thus in figure 84 the thread at the right is shorter, as shown by the break in outline which marks the distal ends of both M-chromosomes, connected here by a thread. No small chromosomes were visible in this case. It is quite possible that such inequality is due to the interpolation of the latter be-

tween the M-chromosomes and the X-chromosome, a fact which stands out very clearly during the dissociation of some of the complexes. The same could be said of figure 83. Here a small portion of one of the small chromosomes is seen to the left of the X-chromosome. The M-chromosome to the right is shorter than that to the left, and a break of outline suggests that this inequality is also due to the interpolation of the other small chromosome, otherwise apparently lacking. It might be well to emphasize that this peculiarity was discovered some time after the figures were drawn, when the intermediate position of the small chromosomes in the dissociating complex was definitely established.

During the pachytene stage the M-chromosomes are approximately as thick as the euchromosomes. However, the latter are double, as shown by the splitting which appears during the short diplotene condition. I think there can be no doubt about the univalent character of the M-chromosomes, for if we consider them as double, their number in the spermatogonia should be four instead of two, an open contradiction with the facts. Assuming that the M-chromosomes are ordinary chromosomes, as indicated by their structure throughout the growth period, I thought ('14) there was evidence in favor of telosynapsis for the euchromosomes. The splitting of the diplotene stage was regarded as the plane of the equational division, which was already present even before the first division had taken place. I am more inclined at present to admit the existence of parasynapsis, although there is not conclusive evidence supporting this view.

If parasynapsis really occur, we cannot fail to recognize that the behavior of the M-chromosomes in this respect is entirely different from that of the euchromosomes. Furthermore, the direct connection of the X-chromosomes and the interpolation of the small chromosomes between the latter and the X-chromosome removes the possibility of a normal parasynaptic association, or at least makes it very problematical.

The importance of the behavior of the M-chromosomes for the interpretation of the complex will be discussed elsewhere.

V. DISCUSSION

1. *The growth period*

There are two points in the growth period of *Blaps* which, I believe, deserve special attention, inasmuch as they are highly characteristic of this species. One is the presence of a resting or confused stage connecting the anaphase of the last spermatogonial division with the prochromosome stage. The other is the occurrence of synizesis before the unraveling stage. Both conditions, already described in my earlier papers, have been found in all the individuals studied so far, irrespective of the fluids used in the preservation of the material, and therefore seem to be entirely normal.

The massive prochromosomes have been found in several forms in both animals and plants, and in many cases appear to be derived directly from the anaphase chromosomes, without an intervening confused stage. But in other instances the telophase of the last spermatogonial mitosis may go so far as to produce a coarse network in which it is impossible to detect individual chromosomes. Such a condition has been described by Professor Wilson in *Lygaeus* and *Oncopeltus* ('12); in the former, cells with a nuclear network occur together with spermatogonial anaphases within the same cyst. However, it could not be proved whether or not the latter were those of the last spermatogonial division. On the other hand, it was impossible, too, to trace step by step the origin of the massive prochromosomes, "for there is no way of demonstrating the seriation at this time, and the change is probably effected rapidly" ('12, p. 367).

We have in *Blaps* a clear case in which the prochromosomes arise from a network, as proved by the seriation of the stages, very easily seen in this form, and by the fact that the origin of such bodies could be traced even within the same cyst. The evidence seems to be conclusive, if we keep in mind that both conditions are occasionally found within the same cyst and that the seriation of later stages, on the one hand, and the conspicuous difference in size between the cells in both stages, on the other, do not warrant another interpretation.

The presence of massive prochromosomes is not of general occurrence among the Coleoptera. While it has been possible to find in *Blaps* hundreds of cysts in such a condition, it is evident that they are lacking altogether in other species. Miss Stevens ('06, '09), who studied more than forty species of beetles, found them in but two species—*Photinus pennsylvanicus* and *Limoneus griseus*. She maintained that they are traceable directly to the chromosomes of the anaphase; indeed, they are but those chromosomes which have retained their massive condition for some time. Arnold ('08) has described similar bodies in the early spermatocyte of *Hydrophilus piceus*, and states that each of them represents a somatic chromosome, although he gives no definite proof of this assertion. According to the descriptions of this author, there is not a confused stage between the last anaphase and the prochromosomes.

Owing to the presence of such a confused stage in *Blaps*, we face here a serious difficulty in assuming that the prochromosomes are the representatives of the chromosomes of the spermatogonia. If there have been sharp criticism of this view when they arise directly from such chromosomes, still more skepticism will arise when considering that their individuality is apparently lost in a coarse network. We have, however, strong reasons favoring the former view, since the number of prochromosomes agrees with the diploid number and the large chromosomes can be recognized as separated individuals during this stage. I wish, however, to emphasize a point which stands out clearly in studying the chromosomes of *Blaps*. This is the remarkable plasticity of the chromatin which leads to surprising changes of shape, if not of volume, in the individual chromosomes. This could hardly be due to the effect of the reagents, since it appears in material preserved by different methods. As a result, it is very difficult to establish constant individual differences among the euchromosomes.

The way in which the prochromosomes arise in *Blaps* suggests a profound reorganization of the chromatin within the prochromosome, a process which might account for the considerable duration of this stage. It is interesting to note that no threads, such

as those present in the somatic prophase, precede the prochromosomes and that the process which I have ventured to call 'pseudo-prophase' seems to be unique in the history of the germ cells. But it is still more interesting when we compare such massive bodies with others, somewhat similar, which occur in the early prophase of the spermatogonia. In these cells the prophase begins with the appearance of thick bands of chromatin, of ragged outline and segmentated appearance (figs. 8 and 9). Near cells with such structure are others which contain nuclei with thin threads (fig. 10, *a*). A comparison of the two conditions compels us to admit either that the thick bands represent a stage preceding the thick threads or that there are two processes by which the formation of the prophase chromosomes takes place.

The last assumption is not supported by any evidence. On the contrary, there seems to be some proofs in favor of the first view in the conditions described in the spermatogonial prophases of Triton by Janssens ('01) and in those of *Phrynotettix* by Pinney ('08) and Wenrich ('16). I have failed to find a conclusive proof of such facts in Blaps, although conditions observed strongly point to it. It is possible that the same phenomena, carried to a greater extent, occur in the prochromosomes, for such bodies begin as a mass of chromatin granules and end as a tightly coiled thread.

Perhaps the extent to which this internal reorganization of the chromosomes is carried may be the cause of the apparently precocious synizesis, which also is highly characteristic of Blaps. In other animals and plants the synaptic knot is formed after the leptotene stage is reached or, at least, when the unraveling of the chromosomes is well advanced. On account of the extreme delicacy of the leptotene threads, considerable doubts have been expressed as to the normal occurrence of such contraction, which has been thought by some authors to be an artifact due to the action of the reagents. It seems unnecessary to discuss this point further, since it has been shown that synizesis also takes place in the living cell. Whatever the origin of synizesis may be, it is probably based on a definite phenomenon, the nature of which we cannot understand at present. It might be well to

emphasize that it appears in my material irrespective of the preserving fluids used, but that individual differences are conspicuous. Without denying some influence on the part of the reagents—e.g., length of preservation, shrinkage due to the embedding process, etc.—the gradual shifting of the massive pro-chromosomes toward a particular pole of the nucleus is a fact which, I hope, will not fail to impress the mind of any impartial observer.

2. The X-complex

The interpretation of the X-complex is at present an exceedingly difficult question. None of the oogonia in mitosis being available, definite knowledge of the number of chromosomes in this sex is lacking. This information is of the utmost importance, inasmuch as the X-complex of this form appears as an intermediate condition between such complexes as are made up exclusively of sex chromosomes and those arising by the linkage of one of the latter with a pair of euchromosomes. The data furnished by the study of the conditions in the female will doubtless settle this question, since in the first case we should expect to find an exact duplication of the group of chromosomes entering into one of the secondary spermatocytes, whereas, in the second, only the dense chromosome which appears as a massive body during the growth period would be double.

The view that the X-complex of *Blaps* is formed by the association of the X-chromosome and a pair of euchromosomes was adopted, too hastily perhaps, in my first paper. Further study showed that in several cases a pair of smaller euchromosomes was also linked with the X-chromosome. On account of the considerable number of metaphases showing this linkage, I was led to believe that such association might well be the normal condition; that the failures of linkage, much less frequent, might be regarded as abnormalities of synapsis ('15, p. 182).

Since evidence enough to support this assumption is lacking, no further attempt to maintain this view will be made here. It seems better to emphasize the differences between the complex under consideration and the conditions observed in other forms,

inasmuch as on these differences hinges our whole interpretation of the facts.

We might adopt the view that the four chromosomes entering one of the secondary spermatocytes constitute a multiple X-chromosome acting like a unit, while the M-chromosome passing to the other spermatocyte is the so called Y-chromosome; but this encounters serious difficulties. Not the least of these is that we have no conclusive proof that the chromosome thus segregated from the complex is constantly one and the same individual. In the event of marked differences between the M-chromosomes, it would be easy to decide on this point, but the observed differences are far from being constant. Without denying the above-suggested possibility, we must not overlook another one, namely, that the distribution of the M-chromosomes among the spermatocytes may be a matter of chance, as proved for heteromorphci pairs of euchromosomes in some Orthoptera (Carothers, '13, '17; Voinov, '14; Wenrich, '14, '16; Robertson, '15).

This interpretation is strengthened to some extent by the behavior of the components of the X-complex during the growth period. We cannot understand at present why one of the chromosomes is condensed, showing all the typical features of the sex chromosomes of other animals, while the others closely resemble the conditions of the euchromosomes. Wherever it has been possible to trace the history of a double or multiple X-chromosome, it appears—with the possible exception of *Ascaris lumbricoides* (Edwards, '10) and *Ascaris incurva* (Goodrich, '16)—as a group of massive bodies throughout the postsynaptic stages, whether or not associated with a Y-chromosome. It is found in this condition in the spiders (Berry, '06; Wallace, '05, '09; Painter, '14) and in the complexes of some Hemiptera—*Fitchia*, *Rocconota*, *Conorrhinus*, *Prionidus*, *Sinea* and *Acholla* (Payne, '09, '10). In other species they fuse into a single mass in which no individual chromosomes can be detected, as occurs in *Galgulus* (Payne, '08), *Syromastes* (Gross, '04; Wilson, '11) and *Ascaris canis* (Walton, '16). Furthermore, the Y-chromosome, when present, appears always as a massive body. The only exception to this rule is, if I remember correctly, the case of the

hemipteron *Enchenopa* (Kornhauser, '14). Perhaps not much stress should be laid on this point since, as a whole, the behavior of the complexes during the growth period is not very accurately known.

The facts just mentioned are in remarkable contrast with the conditions in *Blaps* and point to another interpretation of the X-complex in the latter case. But we cannot fail to recognize that in *Blaps* the assumption of a linkage of the X-chromosome with two pairs of euchromosomes of unequal size also meets with important objections. The failure of the M-chromosomes, and, probably also, of the small chromosomes, to pair at synapsis, or, at least, to remain paired during the postsynaptic stages, is a noteworthy feature which departs from the conditions described in other forms. If we regard the M-chromosomes as euchromosomes, we must accept the fact that they are of a peculiar kind. I must confess that definite proof showing that the M-chromosomes do not undergo synapsis is lacking at present; indeed, the actual pairing of any chromosomes was never observed in my slides, and the assumption that parasynapsis occurs is merely an inference based on the behavior of the bivalents during the diplotene stage. We can be sure, however, that, in the event of a parasynaptic association of the M-chromosomes, this condition lasts but a short time, since their independence is well shown in the early pachytene nuclei.

It is true that this peculiarity in itself does not give a strong ground for either accepting or rejecting the idea that we are dealing with a pair of euchromosomes linked with the sex chromosome. But since no explanation of the nature of the X-complex is given or even suggested in the present paper, the statement has no other value than the recognition of a fact, the nature of which is still unknown. Further, such a condition is of some theoretical interest, since it proves in an unmistakable way the absence of crossing over for the two sets of hereditary factors located in the M-chromosomes, which, if we judge from the considerable size of the latter, must involve an extensive series of characters.

In the complexes of *Hesperotettix* and *Mermiria* (McClung, '05, '17), which may be considered as the type of their class, the euchromosomes which are linked with the sex chromosome behave in a normal way, namely, they appear in the prophase, forming a tetrad which suggests a parasynaptic association in earlier stages. The same is true in the case of some specimens of *Chorthippus curtipennis* (Robertson, '16) and also in *Gryllotalpa vulgaris* (Voinov, '14) judging from the figures given by this author.

In *Hesperotettix* and *Mermiria* the linkage of the X-chromosome with one euchromosome can be detected already in the spermatogonia, a condition most likely to be found in *Leptynia* (De Sinéty, '01), judging from the even number of chromosomes represented in the figure given by this author. The same relations are also present in *Anopheles* (Stevens, '10, under *Culex*; '11) in which an XY pair of sex chromosomes occurs, and still more conspicuously in *Ascaris megalocephala* (Edwards, '10; Frolowa, '12), although in the latter the sex chromosome may occasionally be free (Boring, '09), a condition also found in some individuals in both *Hesperotettix* and *Mermiria*.

Since three V-shaped chromosomes exist also in *Blaps*, I have not overlooked the possibility of a similar linkage. Unfortunately, the most impartial and careful study of my slides fails to show any trace of linkage whatsoever; neither breaks in outline nor differences of structure or staining capacity in the limbs of the V-shaped chromosomes can be detected in this species. The assumption of a linkage of a sex chromosome with an euchromosome, instead of explaining satisfactorily the question at issue, would render it still more complicated in view of the odd number of chromosomes involved and the failure of parasynapsis. It seems plain that the continuity of the three large spermatogonial chromosomes with the large massive bodies of the anaphase of the first maturation division is a fact beyond any doubt.

There is still another point which deserves special attention, and is by no means explicable at present. This is the distribution of the small chromosomes among the secondary spermatocytes, which gives rise to cells with eighteen and seventeen

chromosomes, respectively. As already mentioned, the normal or, perhaps better, the usual condition is that the small chromosomes pass along with the X-chromosome into the same cell. How the former distribution is brought about I do not know. It is an open question whether or not such cells give rise to functional spermatozoa. If they do, slight numerical variations ought to be found in certain individuals. We have ample proofs on the viability of individuals with a number of chromosomes slightly different from the normal in the species, as furnished by the cases in which supernumerary chromosomes occur, and also by the remarkable case of non-disjunction of the sex chromosomes in *Drosophila* (Bridges, '16). But, in all the individuals of *Blaps* so far studied, the number of chromosomes appears to be constant.

Some of the figures on the dissociation of the complex might convey the impression that occasionally the X-chromosome takes the place of one of the M-chromosomes and enters one of the secondary spermatocytes alone. Without denying this possibility, on account of the striking numerical variations observed here and there, I must emphasize the variable position of the X-chromosome with reference to the M-chromosomes. It is evident that in some cases this chromosome is not at the apex, but in the free end of one of the arms of the V-shaped complex. Therefore the M-chromosome, which appears occupying the apex passes alone into one of the spermatocytes, while the others enter the opposite cell. Under identical conditions the normal distribution may also take place, the X-chromosome, and the M-chromosome to which it is linked, entering the same cell. These facts strongly suggest the possibility of a variation in the fiber attachment, leading to conditions very different from those described in the metaphase and pointing to an efficient distribution of the components of the complex. In teleological language, the most important thing here would be, not the relative position of these components, but their final distribution, and in order to fulfill this end a corresponding variation in the attachment of the spindle fibers takes place.

It is quite possible that the complex under discussion is present only in some individuals and that the primitive condition would not show such association, the X-chromosome being free as in the vast majority of the beetles hitherto studied. With regard to this point, it seems necessary to emphasize the fact that all my specimens have been collected almost in the same spot and that their number is not considerable, as such conditions ought always to be taken into account in critical cytological research. I do not know, therefore, whether the inspection of individuals from other localities would lead to the same conclusions. It is interesting to note that in *Blaps waltli*, a closely related but sharply distinct species, a similar complex occurs and that the behavior of this complex during the growth period and first maturation mitosis is the same, although the number and size relations of the chromosomes involved is slightly different. Since I have slides from only three individuals of this species and the technique is not specially favorable for a detailed study, its description has not been included here.

The differences between the X-complex of *Blaps* and those hitherto known, suggest that we are probably dealing with a condition of the utmost importance in the history of the germ cells, closely related with the evolution of the sex chromatin. The behavior of the chromosomes of the complex gives the impression either that a group of euchromosomes is in the course of secondary association with the sex chromosome or that a large composite X-chromosome is breaking up into several bodies which have not yet attained to complete independence. Which of the two processes is taking place we cannot say at present.

This case is comparable to the interesting XY complex of *Notopecta indica*, described by Miss Browne ('16), in which the X-chromosome is composite, appearing at the prophase of the first maturation division as a central dense mass and two lateral threads in which an unequal number of knobs occur. Such a condition is regarded by the author as a step in the liberation of chromatin from the sex chromosome. A further step would be represented by *Blaps* in which the chromosomes have already freed themselves, but still lack the characteristics of the euchro-

mosomes, so far as synapsis is concerned. But such a conclusion would be of very hypothetical character and is without adequate support in the facts observed elsewhere.

VI. CONCLUSIONS

1. The spermatogonial groups of *Blaps lusitanica* contain thirty-five chromosomes, thirty of which pair at synapsis, giving rise to fifteen bivalents in the primary spermatocytes. The other five chromosomes join in a group, the X-complex, during the early synizesis and can be traced from this stage to the metaphase of the first maturation division.

2. The chromosomes of the X-complex differ in size and shape, three of them being much larger than two others which cannot be distinguished from the ordinary chromosomes in the spermatogonia. The large chromosomes appear in the latter as atelomitic V-shaped bodies. Two of them, which appear to be homologous and have been termed M-chromosomes, have a submedian attachment of the spindle fibers, while the third has a subterminal attachment: The latter element, as shown by its behavior during the growth period, corresponds to the accessory or X-chromosome of other forms.

3. A resting stage intervenes between the last spermatogonial mitosis and the prochromosome stage. The prochromosomes appear as massive bodies, their number agreeing with that of the spermatogonial groups.

4. Synizesis takes place when the chromatin is still in the form of massive prochromosomes.

5. During the unraveling stage the M-chromosomes and small chromosomes of the X-complex give rise to leptotene threads, whereas the X-chromosome retains its dense condition.

6. The M-chromosomes, and probably also the small chromosomes of the X-complex, do not pair at synapsis, but persist throughout the pachytene stage as independent threads connected with the X-chromosome. Their relative position in the complex is variable.

7. The X-complex undergoes dissociation during the anaphase of the first maturation mitosis. One of the M-chromosomes

segregates from the others and passes into one of the secondary spermatocytes, which receives sixteen chromosomes (15 euchromosomes plus 1 chromosome of the X-complex). The other spermatocyte receives nineteen chromosomes (15 euchromosomes plus 4 chromosomes of the X-complex).

8. There is no conclusive evidence of predetermination in the segregation of the M-chromosomes during the reduction division. It is possible that their distribution with reference to the X-chromosome follows the law of chance.

9. The chromosomes of the X-complex are separate in the secondary spermatocytes and the two classes of the latter are found in about equal numbers.

10. The second maturation mitosis is equational.

11. The X-complex seems to represent an intermediate condition between complexes made up exclusively of sex chromosomes and those originated by the linkage of a sex chromosome with a pair of ordinary chromosomes.

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EXPLANATION OF PLATES

The figures in all the plates were drawn with the aid of the camera lucida, using in every case a Zeiss hom. imm. 1.5 mm. lens, the oculars varying according to the size of the cell. In figures 1 to 15, 22, and 27 to 35 a Zeiss compens. ocul. No. 12 was used, giving a magnification of 2950 diameters. The same combination was used for figures 16 to 21, 23 to 26, 39, 40, and 42 to 63, but they were subsequently enlarged $1\frac{1}{2}$ diameters to work out details. Figures 36 to 38 and 66 to 86 were made using a Spencer compens. ocul. No. 20, at a magnification of 3600 diameters. Figure 64 was drawn with Spencer ocular 6.

All figures have been reduced $\frac{1}{2}$ in reproduction.

PLATE 1

EXPLANATION OF FIGURES

1 to 6 Nuclei of secondary spermatogonia showing individualized chromosome X in different conditions of condensation.

7 The same chromosome drawn from three nuclei belonging to secondary spermatogonia.

8 and 9 Early spermatogonial prophase showing chromatic bands.

10 and 11 Two sections of two spermatogonia showing the orientation of the large chromosomes within the nucleus.

12 Prophase in the spermatogonia of latter generations.

JOSE F. NONIDEX

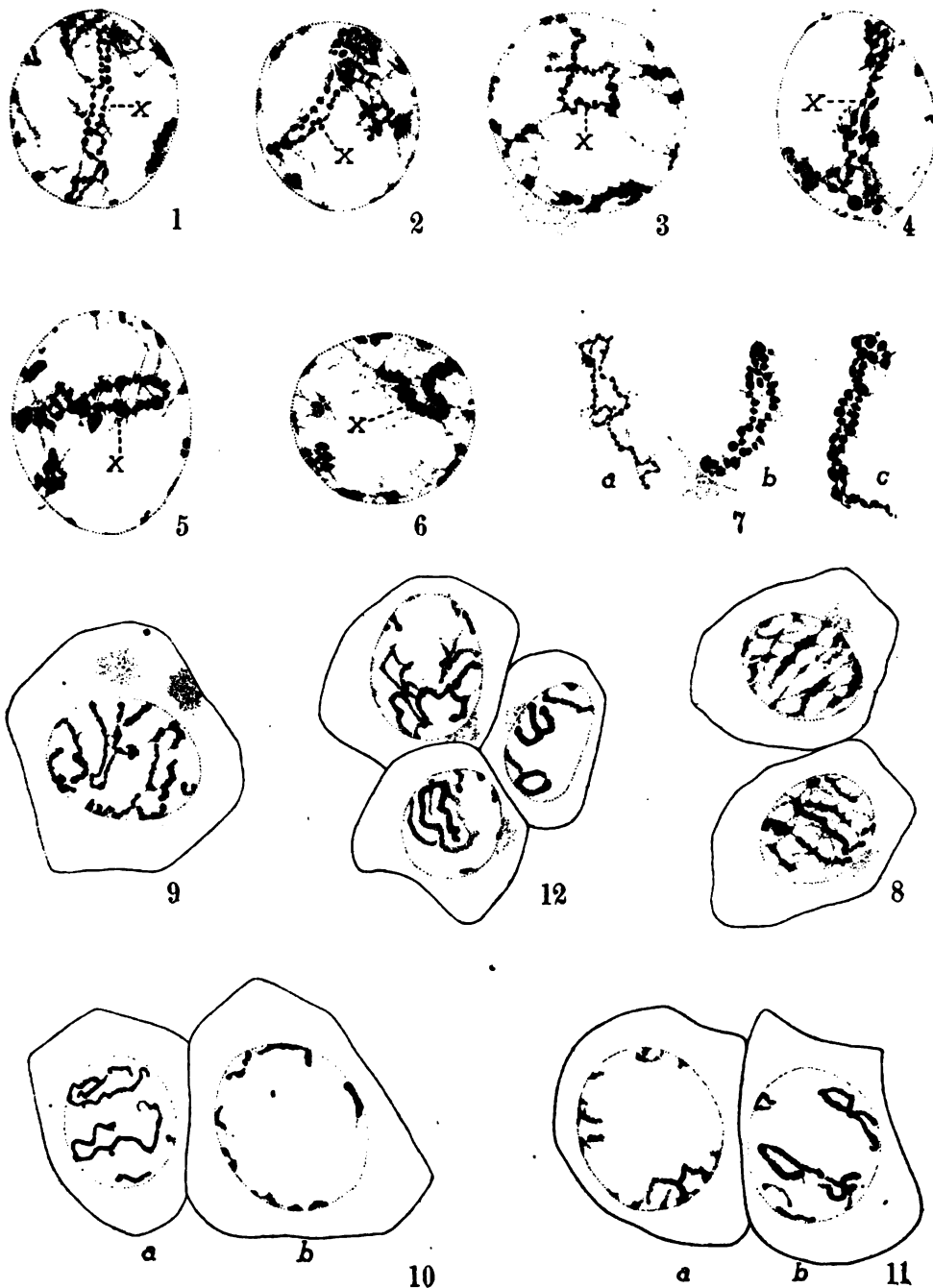


PLATE 2

EXPLANATION OF FIGURES

13 to 15 Three stages of the late prophase in the secondary spermatogonia.

16 to 20 Metaphases of secondary spermatogonia showing the X-chromosome (X) and the M-chromosomes (M, M'). Figures 16, 19 and 20 belong to the same individual, but to different cellular generations.

21 Large chromosomes drawn from spermatogonia of the same individual to show variation in shape and size. The chromosomes represented in *f* have been drawn from one of the last spermatogonial mitoses.

22 Metaphase of secondary spermatogonium seen in lateral view; the chromosomes are split lengthwise.

23 to 26 The large chromosomes as seen in the early anaphase of secondary spermatogonia, showing differences in the mode of attachment of the spindle fibers.

JOSE F. NONIDEE

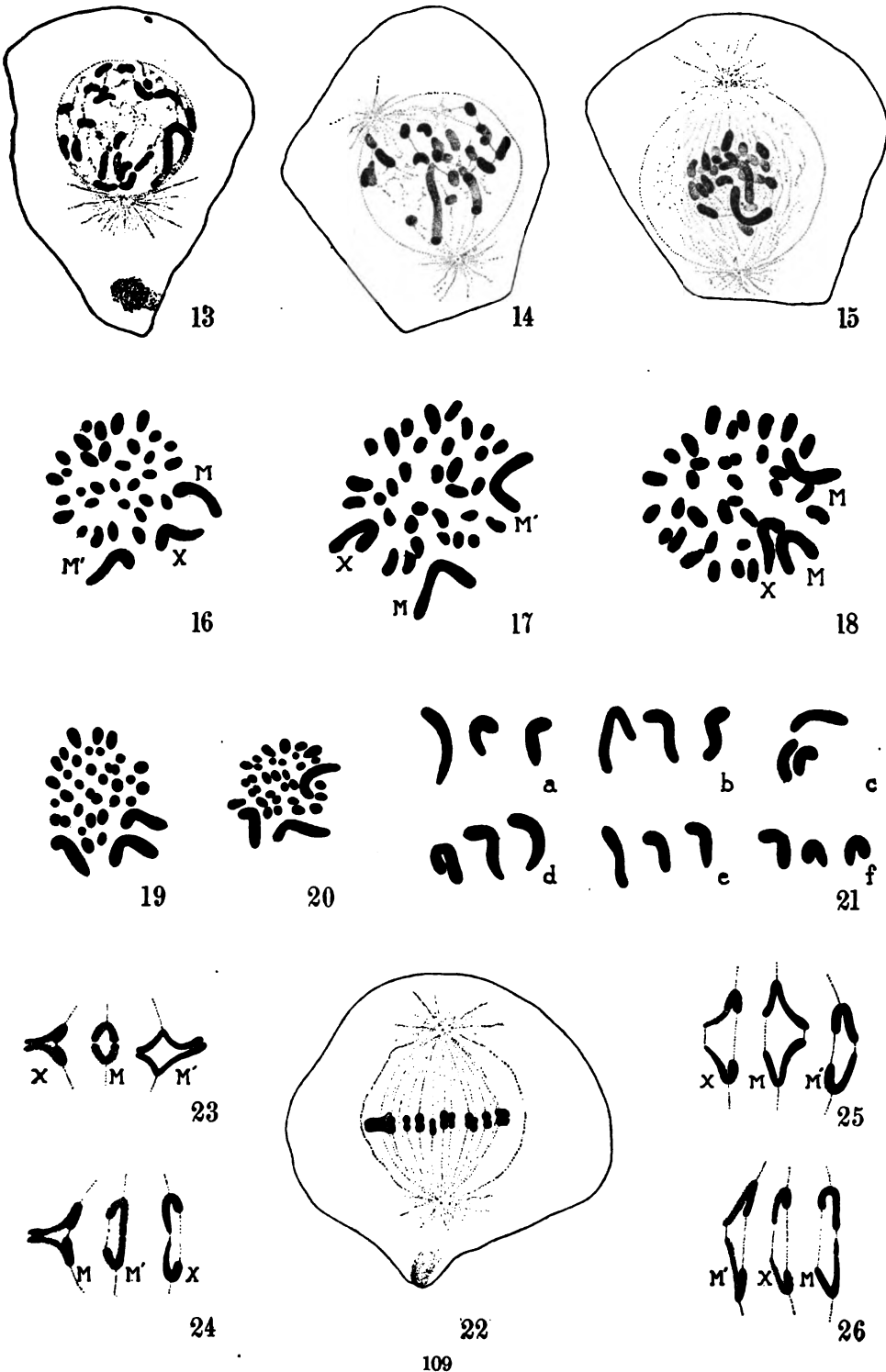


PLATE 3

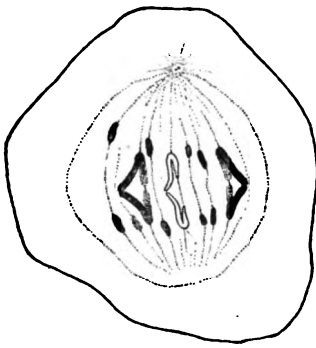
EXPLANATION OF FIGURES

27 Early anaphase of a secondary spermatogonium showing differences in the attachment of the spindle fibers.

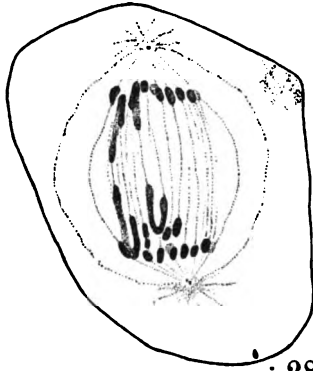
28 to 31 Later stages in the anaphase of secondary spermatogonia.

32 to 34 Telophases showing migration of the centrosome.

35 Advanced telophase of secondary spermatogonium. The mitosome or spindle remnant is arising and the centrosome (only seen in the upper cell) has completely moved around the nucleus.



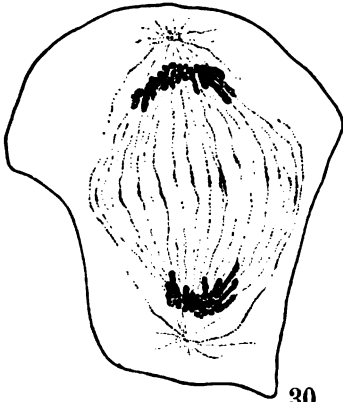
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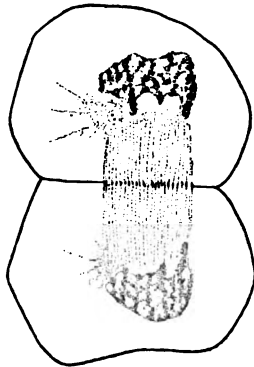
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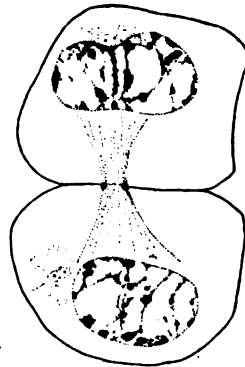
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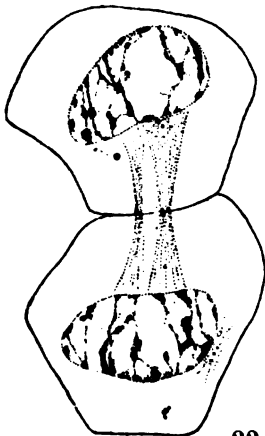
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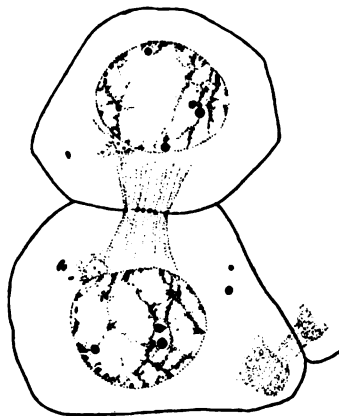
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32



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34



35

PLATE 4

EXPLANATION OF FIGURES

36 Four sections of the nucleus of a spermatocyte during the prophase of the first maturation division, showing the X-complex and fifteen bivalent euchromosomes.

37 Same stage; all the chromosomes represented in the same plane. The condensation of the M-chromosomes is not so advanced as in the preceding figure.

38 Spermatocyte in the early prophase showing splitting of the euchromosomes, which have been disposed in a series in figure 39, according to their degree of condensation.

40 Euchromosomes from different spermatocytes showing differences in the process of splitting. The chromosomes represented in *b* and *d* are Y-shaped chromosomes seen endwise.

41 X-complexes drawn from cells in the early prophase. The M-chromosomes are united directly with each other in *f*, the X-chromosome occupying the end of one of the arms of the V-shaped complex.

42 to 45 Metaphases of the first division in polar view.

46 Four optical sections of a metaphase in lateral view, showing attachment of the spindle fibers in the X-complex.

47 Polar view of a metaphase in which the X-chromosome has failed to condense, appearing as a U-shaped body.

48 Three complexes drawn from metaphases. The X-chromosome has failed to condense in *b*. In *c* a single small chromosome is seen.

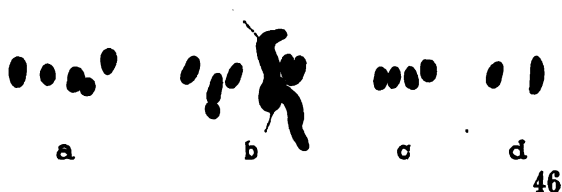
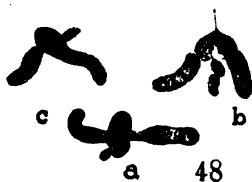
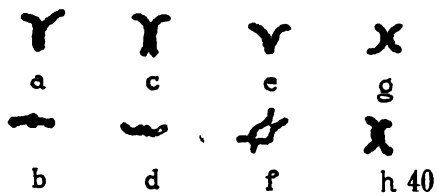
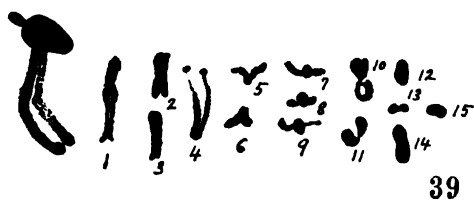
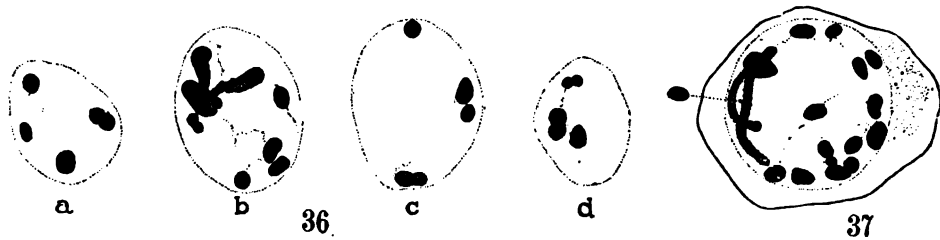


PLATE 5

EXPLANATION OF FIGURES

49 to 51 Anaphases of the first maturation mitosis showing the dissociation of the X-complex and the unequal distribution of its components.

52 and 53 Lateral and polar view of the pole of the spindle receiving the X-chromosome. The large chromosomes, which lag behind the euchromosomes, have been represented in the same plane in figure 53.

54 Several stages of the dissociation of the complex showing the progressive appearance of the small chromosomes.

55 Dissociation of the complex. One of the spindle fibers is attached to one or both small chromosomes.

56 Normal metaphase of a secondary spermatocyte with X-chromosome.

57 Metaphase of a secondary spermatocyte with eighteen chromosomes.

58 and 59 Metaphases of secondary spermatocytes without X-chromosome, with sixteen and seventeen chromosomes, respectively.

60 to 63 Lateral views of the anaphases of the secondary spermatocytes, showing the division of the large chromosomes of the X-complex.

64 Cyst with primary spermatocytes during the resting stage which follows the last spermatogonial mitosis and spermatocytes in the prochromosome stage.

65 Spermatocyte during the resting stage.

66 Formation of the prochromosomes.

67 to 69 Spermatocytes during the prochromosome stage. The X-chromosome is still condensed in figure 67, while in figures 68 and 69 it appears as a coarse thread.

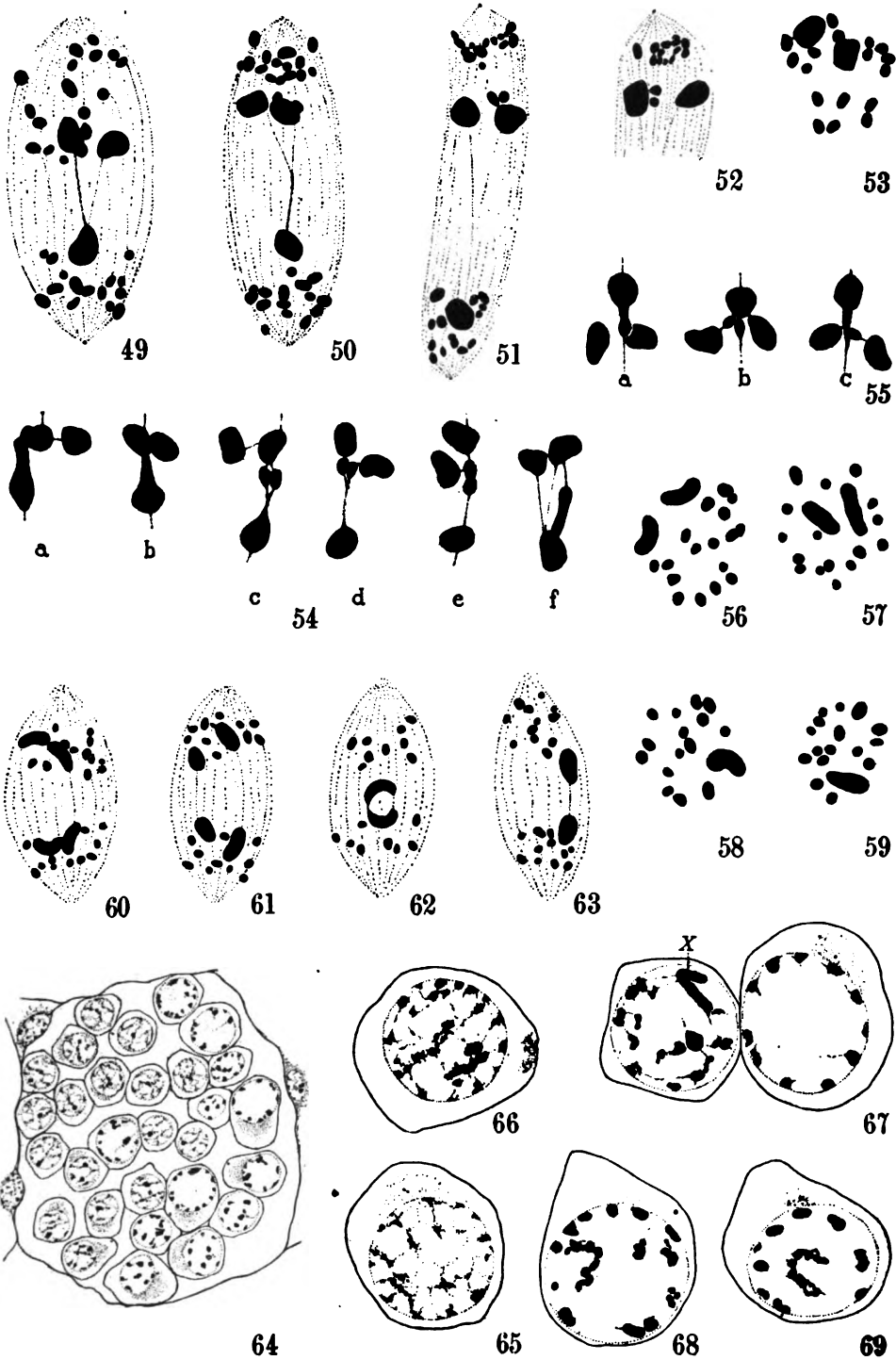


PLATE 6

EXPLANATION OF FIGURES

70 Four optical sections of a primary spermatocyte during the prochromosome stage.

71 Beginning of synizesis. The X-complex (X) is already formed and lags behind the euchromosomes.

72 Two cells during synizesis, showing the condensation of all the prochromosomes in the pole of the nucleus nearest to the sphere; in the cell to the left the X-complex has been represented.

73 Polar view of a spermatocyte during synizesis.

74 X-complexes of spermatocytes during synizesis; the M-chromosomes will soon begin to unravel and their outline appears a little ragged.

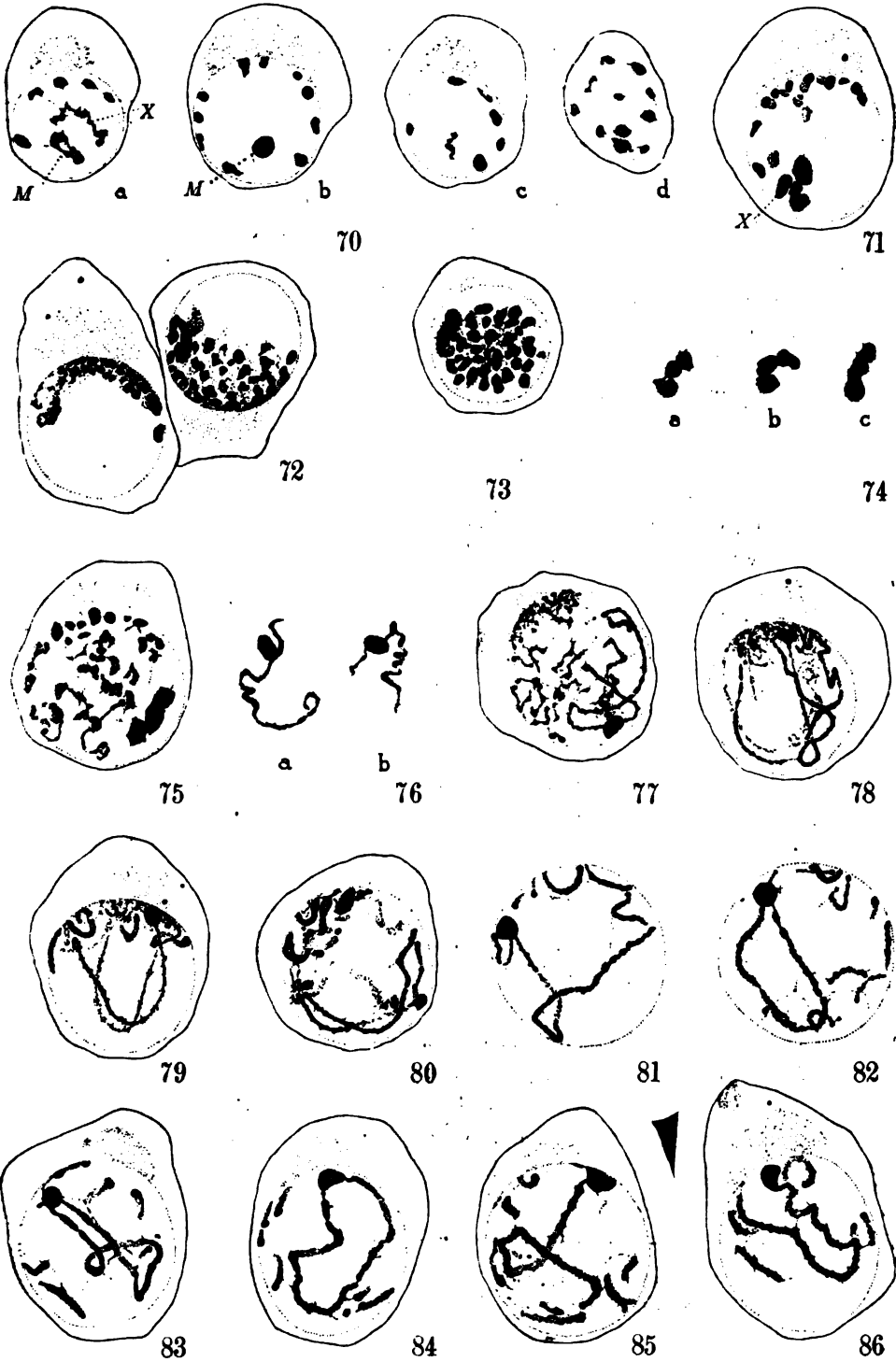
75 Polar view of a spermatocyte at the beginning of the unraveling stage. The X-complex appears conspicuously.

76 Two details of the unraveling of the M-chromosomes.

77 and 78 Leptotene stage, as seen in polar and lateral views of the cells.

79 and 80 Early pachytene stage.

81 to 86 Pachytene stage proper.



Resumen por el autor, Edward L. Rice.
Universidad Wesleyana de Ohio.

El desarrollo del cráneo en *Eumeces quinquelineatus*. L.
I. El condrocráneo.

1. El desarrollo del condrocráneo coincide en general con el desarrollo del condrocráneo de *Lacerta*, descrito por Gaupp, pero se presentan las notables diferencias siguientes: a) aumento de tamaño de la porción coclear de la cápsula ótica, con desplazamiento del orificio facial que adopta una posición intercapsular, lo cual recuerda la condición presente en los mamíferos, corroborando las ideas de Gaupp sobre la invasión progresiva de la placa basal por la coclea; b) oclusión mas definida de la fenestra coclear por tejido conjuntivo y aparentemente homología mas exacta con la membrana timpánica secundaria de los mamíferos; c) imperfección de la pared lateral de la región temporal, debida a la degeneración precoz de ciertos elementos y desarrollo tardío de otros; d) reducción del piso nasal; e) tamaño extraordinario de las ventanas superiores de la nariz; f) ausencia de ventanas laterales de la nariz, que tal vez estén desarrolladas en estados ulteriores. 2. La superficie condilar en forma de media luna, como en el embrión de los mamíferos. 3. Notocordio en su mayor parte dorsal a la placa basal. 4. Ventana basicranial posterior grande. 5. El techo posterior aparentemente de origen occipital, al menos en parte. 6. Columela auditiva interpretada provisionalmente como una formación originada en un estroma embrionario, que se extiende sin interrupción desde el arco hioídeo a la cápsula ótica; la columela está por consiguiente relacionada genéticamente con el arco hioídeo y la cápsula ótica, pero, sin embargo, representa una unidad genética. 7. Fenestración progresiva de los tabiques interorbitario y nasal. 8. Piso supraseptal par. 9. El cartílago palatoptérigoc cuadrado continuo en los anfibios, desmembrado en procesos maxilares, proceso pterigoídeo (probablemente también en el cartílago articular del pterigoides), epipterigoídeo, y cuadrado junto con articular del pterigoides), epipterigoídeo, y cuadrado junto con otros fragmentos variables y aislados. 10. Se han notado variaciones en la salida de los siguientes nervios craneales: VI, IX (a veces "intracapsular," otras veces "etracapsular") y XII (dos o tres orificios; raíces nerviosas mas numerosas).

Translation by José F. Nonides
Cornell University Medical College, N. Y.

THE DEVELOPMENT OF THE SKULL IN THE SKINK, *EUMECES QUINQUELINEATUS* L.

I. THE CHONDROCRANIUM

EDWARD L. RICE

Ohio Wesleyan University

TWELVE PLATES (THIRTY-THREE FIGURES)

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1. INTRODUCTION

Since the publication, in 1900, of Gaupp's classic paper on the chondrocranium of *Lacerta* there have been great additions to the accurate and detailed knowledge of the embryology of the vertebrate skull. But the data for comparative study are still inadequate, and many theoretical questions are still dependent for their satisfactory settlement upon the investigation of a more extensive series of species.

Especially is it through the investigation of closely related forms that the essential and general can be freed from the incidental and individual and built up into a firm foundation for legitimate theorizing. Thus *Eumeces* has a special claim for consideration because of its comparatively close relationship to *Lacerta*, which has become the standard for comparison, thanks to the masterly work of Gaupp.

The similarities and contrasts of *Eumeces* and *Lacerta* may be emphasized most conveniently by a strictly comparative treatment based upon Gaupp's paper ('00). For the most part, both the order of treatment (except for the division of Gaupp's paper into two parts, descriptive and theoretical, respectively) and the terminology of Gaupp are adopted here.

Partial results of this study have been published in the form of brief preliminary notes ('11, '14); here they appear in more complete form. In the present paper the history is followed only to the stage of maximum development of the chondrocranium; the fate of the cartilages in later embryos and in the adult skull and the development of the bones, aside from merely incidental references, are left for later treatment.

The earlier literature on skull development has been so admirably summarized by Gaupp in Hertwig's 'Handbuch' ('05 b) that any further extensive review would be superfluous.

The present investigation was commenced under the supervision of the late Prof. Ernst Gaupp in the Comparative Anatomic Laboratory of the University of Freiburg, Prof. Robert Wiedersheim, director. I wish at this time to express my hearty appreciation of the courtesies, scientific and personal, which were extended so freely during my stay in Freiburg by Professor Wiedersheim, Professor Gaupp, and the other members of the staff.

During the later prosecution of the work I have enjoyed the hospitality of the Lake Laboratory of Ohio State University, the Zoological Laboratory of Harvard University, and the Marine Biological Laboratory at Woods Hole. My thanks are due to Prof. Herbert Osborn, Prof. E. L. Mark, Prof. Frank R. Lillie, and their associates for their many favors.

I have also been materially assisted by several of my advanced students in Ohio Wesleyan University, and would especially thank Mr. Joseph I. Taggart, Miss Margaret Cole, and Mr. Clarence L. Turner for the use of models and unpublished work freely placed at my disposal.

And, finally, I wish to record my indebtedness to the Ohio Academy of Science for a grant from the Emerson McMillin Research Fund, which has provided technical assistance in the preparation of drawings and in the work of modeling. Through this grant I have been able to avail myself of the services of Miss Mabel L. Hedge, whose artistic and accurate drawings are reproduced in figures 1 to 8, inclusive.

2. MATERIAL AND METHODS

The material upon which this paper is based consists of a single brood of eggs collected when the embryos were already considerably advanced. They were preserved at intervals during a period of seventeen days. No data can be given as to absolute ages, but the relative ages may be seen from the following tabulation. The measurements of the table were secured, after preservation, by means of a thread laid along the embryo. The length of the head is measured from the tip of the snout to the

posterior margin of the tympanum; the body is measured from the posterior margin of the tympanum to the anus. The measurements of the tail and of the total length are much less reliable than the other data, owing to the fact that the tip of the tail of almost every embryo was damaged in the preparation of the material.

Tabular statement of size and age of embryos studied

STAGE	AGE IN DAYS	LENGTH IN MILLIMETERS			
		Head	Body	Tail	Total
1	x	3.25	6.50	7.00	16.75
2	x + 2	3.50	7.00	7.50	18.00
3	x + 4	4.00	8.00	?	?
4	x + 6	4.50	9.00	?	?
5	x + 9	4.75	9.75	13.50	28.00
6	x + 17	6.00	13.00	20.00	39.00

The modification in form during the seventeen days of development is shown in figures 29 to 31, which are drawn to scale by means of the camera. In stage 1 the chondrocranium is already well mapped out, although much of the cartilage is in a very young condition and should, perhaps, be designated as procartilage or even blastema. Bone formation is merely suggested—pterygoid, extreme anterior end of dental, and very thin dentine layers in the teeth. Stage 2 shows a marked advance in bone formation, and in stage 5 the full complement of membrane bones may be recognized, with the exception of the lacrimal and parasphenoid. The lacrimal appears in stage 6; the parasphenoid is still wanting in this the latest of my embryos. There is no actual replacement of cartilage by cartilage bone until stage 6 is reached, although the bone formation is anticipated in stage 5 by a marked histological modification of the cartilage in many places. Even in stage 6 the replacement of cartilage by bone is only begun, the most conspicuous ossification centers being found in the posterior part of the basal plate, the occipital arches, the posterior parts of the quadrate and Meckel's cartilage, and some portions of the otic capsule and hyoid. In stage 6 the

scales are clearly visible in the epidermis, and the longitudinal stripes of the juvenile color pattern are already conspicuous.

Stage 5, which is in nearly the same stage of development as the 31-mm. embryo of *Lacerta* described by Gaupp, furnishes the foundation for this paper, but earlier and later stages are used freely for comparison.

The material was preserved in picro-acetic and stained with Mallory's triple connective-tissue stain (acid fuchsin + aniline blue + orange G)—a stain which leaves nothing to be desired as regards clearness of differentiation of bone and cartilage from one another and from surrounding tissues.

The following wax plate models have been available for study and comparison:

- a. Stage 1. Membranous labyrinth, modeled by Mr. J. I. Taggart.
- b. Stage 2. Entire skull and first two vertebrae, cartilage only, modeled by Miss Margaret Cole.
- c. Stage 5. Entire skull and first two vertebrae, cartilage and bone, modeled by the author.
- d. Stage 5. Cavity of otic capsule, modeled by Mr. J. I. Taggart.
- e. Stage 5. Membranous labyrinth, modeled by Mr. J. I. Taggart.
- f. Stage 5. Brain and nerve roots, modeled by Mr. Clarence L. Turner. Figure and brief description published by Mr. Turner ('14).
- g. Stage 6. Posterior portion of skull, cartilage only, with nerve origins, modeled by the author.

3. BASAL PLATE AND ASSOCIATED PARTS

1. *Basal plate and occipital condyle*

The basal plate (figs. 1 and 2), belonging alike to the occipital and otic regions, lends itself to separate and continuous description. As in *Lacerta*, the basal plate consists of a dorsally concave floor between the otic capsules and extending from the foramen magnum at the posterior to the fenestra hypophyseos at the anterior. The anterior margin is strikingly thickened to form the crista sellaris (fig. 1, *cr.sel.*), and from the anterolateral corners arise the trabeculae cranii (fig. 2, *trab.*), the rudimentary pilae prooticae (fig. 1, *pi.pr-ot.*), and the basipterygoid processes (fig. 2, *pr.b-pt.*), which will be described in detail later.

The large fenestra basicranialis posterior (fig. 2, *fen.b-c.p.*) commences immediately behind the crista sellaris, and, in the form of an isosceles triangle with backward pointing apex and rounded basal angles, extends back for more than a third of the length of the basal plate. By this fenestra the basal plate is divided in its forward portion into right and left moieties corresponding to the parachordal cartilages of the lower vertebrates. This division is also suggested for some distance further to the posterior by a shallow but distinct ventral groove; but in the posterior portion there is no suggestion of a double origin of the basal plate, either in stage 5 or in any earlier stage.

The basicranial fenestra appears to be of very general distribution in the Reptilia. Gaupp ('05 b) has collected records of its occurrence in Sphenodon, lizards, snakes, and turtles, and this is confirmed in more recent papers for Vipera (Peyer, '12) and Emys (Kunkel, '12 b). The advanced age of the embryos of Dermochelys, Chelonia, and Chelydra studied by Nick ('12) probably accounts for the absence of this fenestra. In the Crocodilia (Parker, '83; Shiino, '14) there is no evidence for its occurrence. Shiino describes an elongated perforation of the basal plate in connection with the forward part of the notochord, but interprets this as due to secondary resorption and not homologous with the fenestra basicranialis posterior.

Gaupp describes the basal plate as octagonal in Lacerta; in Eumeces the form is much less regular, owing largely to the infringement of the much larger cochlear portion of the otic capsule (figs. 2 and 8, *prom.coch.*). In its anterior portion, from about the level of the posterior border of the fenestra basicranialis posterior, there is a striking lateral expansion of the basal plate, which practically surrounds the cochlear capsule and fuses with its medioventral, anterior, and laterodorsal borders. Except for the interruption of the facialis foramen (figs. 7 and 8, *f.N.VII.*), this fusion is continuous from the fissura metotica (fig. 7, *fis.m-ot.*) to the prominentia recessus utriculi (fig. 7, *prom.r.ut.*), with which it is also united. In front of the otic capsule the free lateral margin of the basal plate is rolled outward, but not noticeably thickened (figs. 2 and 19). The edge

may also be traced backward for a very short distance as a projecting crest (fig. 7; figs. 17 and 18, *b.pl'*.) along the ventrolateral limit of the prominentia recessus utriculi. This rolled edge is clearly homologous with the much more conspicuous projecting edge of the basal plate in Emys to which, in its various regions, Kunkel ('12 b) gives the names of crista inferior, crista substapedialis, and crista basipterygoidea. The relation to the trigeminal ganglion is the same; but a connection with the well-developed processus basipterygoideus is not seen in Eumeces, nor has the ridge here any such posterior extension as in Emys. Under the name processus subcapsularis, Shiino ('14) describes a similar structure in the crocodile, and notes that it is also recognizable in Gaupp's figures of Lacerta, but not in those of Schauinsland ('00) of Sphenodon. From the fenestra basicranialis posterior backward the thickened margin of the basal plate is everywhere separated from the otic capsule by the fissura metotica (figs. 7 and 8, *fis.m-ot.*), although this fissure is practically obliterated, for a short distance immediately posterior to the fenestra cochleae, by the contact (not fusion) of a projecting angle of the basal plate and the swelling of the otic capsule to form the prominence of the posterior ampulla (fig. 7, *prom.amp.p.*).

At the posterolateral angles the basal plate connects with the occipital arches (figs. 2 and 6, *oc.*), and, between these arches, its posterior margin is greatly thickened in the condylar region (figs. 2 and 6, *con.*). The conditions here are essentially as in Lacerta, and also essentially as in the embryonic Echidna skull (Gaupp, '08 a). In the median line, at the entrance of the notochord, there is a decided notch (fig. 2, *in.i-c.*). Compare the incisura intercondyloidea of Echidna (Gaupp, '08 a), the rabbit (Voit, '09 b), the dog (Olmstead, '11), and other mammalian embryos. At the sides of this intercondylar notch are two conspicuously projecting domes of cartilage which can hardly be other than the homologue of the mammalian condyles. Ventral and anterior to the notch the condylar projections are connected by a thickening of the cartilage. Thus is formed a continuous crescentic or kidney-shaped condylar mass, from which it is easy to derive the specialized single condyle of the majority of

reptiles and the divergently specialized paired condyles of most mammals. The approach in adult forms of both mammals and reptiles to this undifferentiated type has been emphasized by Osborn, in collaboration with Bensley (Osborn, '00), by Mead ('06), and by Gaupp in several special papers as well as in a general summary before the Anatomische Gesellschaft ('08 b). The plane of my sections is not particularly adapted to the study of the skull articulation, but there can be no doubt that the atlas actually articulates with the entire condylar surface, lateral as well as median. In fact, the emphasis seems to be laid on the lateral portions rather than the median. It seems evident also that the articular capsules, between skull and atlas and between atlas and epistropheus, show no essential departure from the 'monocöler Typus' described by Gaupp ('08 b) as characteristic of the Sauropsida and, with slight modification, as primitive in the Mammalia. Among mammals this form of articulation was first demonstrated by Fischer ('01 a and b) for the embryo of *Talpa*; the discovery was later extended to a variety of other mammals, adult as well as embryonic, by Gaupp ('07 a and b; '08 b) and Grosser ('07). Shiino ('14) states that in *Crocodylus*, as in *Emys* and *Sphenodon*, a single condyle is present, with no suggestion of the lateral extensions seen in *Lacerta*, and Parker's figure of the crocodile ('83, reproduced in Gaupp, '05 b) shows a similar structure. It is interesting in this connection to note that Kunkel ('12 b) shows a rather closer approach to the conditions in *Lacerta* in the earlier embryos of *Emys*, while Fuchs ('12), on the basis of his study of *Chelone* embryos, definitely accepts the reniform condyle as the starting-point alike for the dicondylous of the mammals and the monocondylous of the reptiles. It may well be that a further study of younger stages will show a closer approach to this type on the part of *Sphenodon* and the crocodiles. Vesper (Peyer, '12) apparently agrees with *Lacerta* and *Eumeces*.

In stage 5 of *Eumeces* there is apparently a complete confluence, although with some slight histological differentiation, of the cartilage of the odontoid process and the basal plate (fig. 9,

b.pl. and *pr.odont.*). Miss Cole, working independently upon stage 2, has arrived at the same conclusion concerning the continuity of these cartilages. Even in stage 6 there is still a slight union of the dens with the basal plate, but this is much less extensive and less definite than in the earlier stages. These observations are in accord with the view of Gaupp ('07 b; '08 b, p. 187) that "der Dens epistrophei bei den Amnioten ursprünglich bis auf die Schädelbasis reichte, ja geradezu in seinem vordersten Teil aus Bildungsmaterial der Occipitalregion hervorging." Direct evidence of such a composite character of the dens in various mammals is given by Weiss ('01), Weigner ('11), Terry ('13), and de Burlet ('13 a, '16).

2. *Notochord*

As in *Lacerta*, the notochord passes from the odontoid process to the dorsal side of the basal plate. The continuity of cartilage just discussed makes interpretation somewhat difficult; but I believe that, for a short distance in front of the odontoid process, the notochord is embedded in the cartilage of the basal plate (fig. 6, *ch.*). The dorsal covering is very thin and of slight extent, the notochord soon emerging on the surface (fig. 1, *ch.*). From this point forward to the fenestra basicranialis posterior it lies in a very noticeable groove of the basal plate (fig. 10), embedded in a rather dense but thin fibrous connective tissue, which is continuous with the perichondrium of the basal plate. In the extreme forward part of this course the ventral side of the basal plate shows a similar but less marked median groove filled with a similar fibrous extension of the perichondrium, and suggesting the original paired parachordal character of the basal plate. When the two parts of the basal plate separate at the fenestra basicranialis posterior, this connective tissue remains as the 'filling tissue,' noted by Gaupp, in which the forward end of the notochord is embedded. As in *Lacerta*, the notochord terminates freely in the fenestra basicranialis posterior, just posterior to the crista sellaris.

In stage 5 the notochord must be interpreted as already in degeneration—a process which is much more conspicuous in stage 6, where the anterior portion of the notochord is broken into a series of isolated fragments. A similar fragmentation is described by Gaupp ('08 a) in *Echidna*. In stage 6 the notochord terminates much more posteriorly than in stage 5, while in the earlier embryos, stages 1 to 4, the anterior end is deeply embedded in the cartilage of the crista sellaris. In *Sphenodon* (Schauinsland, '00), *Emys* (Kunkel, '12 b), *Alligator* (Parker, '83), and *Crocodylus* (Shiino, '14) the notochord is described as penetrating the crista sellaris in certain stages and extending into the fenestra hypophyseos. These data practically demonstrate the homology of the crista sellaris of the reptiles and the anterior thickened margin of the basal plate, 'Sattellehne,' in the *Selachii*, questioned by Gaupp ('05 b) because of the more posterior termination of the notochord in *Lactera*. It is interesting to note that Gaupp himself pointed the way to the solution of his question when he suggested (p. 759)

dass die Frage berechtigt ist, ob nicht vielleicht die Lage der Crista vor der Chorda lediglich auf frühzeitigen Schwund des vordersten Chordaabschnittes zurückzuführen ist. Um das zu unterscheiden, müsste das Verhalten der Chorda von frühesten Stadien an bis zur Bildung der Crista sellaris verfolgt werden.

In the earlier stages of *Eumeces* the posterior cartilaginous covering of the notochord is much more marked, while in stage 6 the position of the notochord is more superficial with reference to the basal plate, and no cartilaginous investment can be certainly demonstrated in front of the dens epistrophei. The dorsal covering of the notochord is not noted by Gaupp in *Lacerta*, but may have been present in earlier stages. In *Tropidonotus* (Gaupp, '00) and *Vipera* (Peyer, '12) the notochord is wholly dorsal to the basal plate, as in *Lacerta*; in *Sphenodon* (Schauinsland, '00) and in late embryos and adults of *Dermochelys*, *Chelone midas*, and *Chelydra* (Nick, '12) the course is like that in earlier *Eumeces* embryos, except that the notochord is embedded in the cartilage for a much greater part of its course.

Gaupp ('00) also notes that in *Chelone midas* the notochord enters the basal plate ventrally, in marked contrast to the dorsal position in *Lacerta* and *Tropidonotus*. Finally, in *Emys* (Kunkel, '11, '12 b) and *Crocodylus* (Shiino, '14) the notochord is described as wholly embedded in the basal plate, although Kunkel believes it to have lain on the dorsal surface in earlier stages of *Emys*. This variability in the reptiles finds its parallel in amphibians and mammals. Thus, according to Gaupp ('05 b), in *Triton taeniatus* the notochord lies dorsal and in *Siredon ventral*, while in *Triton cristatus* it is embedded in the basal plate. Gaupp ('08 a), Tourneux ('12), and de Burlet ('13 a, '16) have collated the data for the Mammalia, showing an extreme dorsal position in *Echidna* and *Mus*, a fully ventral position in *Bradypus*, complete investment in *Bos* and *Phocaena*, and a variety of combinations of these types, sometimes very complex, in other forms. The contrasts between closely related species as well as between different ontogenetic stages of the same animal indicate the correctness of Gaupp's view ('08 a) that little significance attaches to the position of the notochord.

3. Nerve foramina of basal plate

The basal plate is intimately associated with the points of exit of two pairs of cranial nerves—XII, hypoglossus; VI, abducens. The foramen for the facialis and the fissura metotica, with its complex of nerves and blood-vessels, can be more conveniently discussed in connection with the otic region.

Nerve XII. The hypoglossus foramina (figs. 2, 3, 6, 7, and 8, *f.n.XII.*) are located essentially as in *Lacerta*, near the lateral edge of the basal plate, the posterior foramen lying just opposite the union of the basal plate with the occipital arch; but the number is only two on each side in stage 5. Other stages of *Eumeces* show variability in this number. Thus in stages 2 and 4 there are two foramina on one side and three on the other (in stage 4 the third foramen is very minute), while stage 6 shows three foramina on each side, as in *Lacerta*.

A careful study of the sections indicates clearly that the number of hypoglossus nerve roots (as marked by independent emergence from the surface of the brain) is more than three. The thickness of my sections makes it unsafe to affirm that all such roots have been identified; the numbers must therefore be taken as minimal. In stage 4 five such origins have been recognized on each side. In general the conditions seem to be much more uniform in the region of the posterior foramen, where a single large root (made up of two subdivisions in stage 4) emerges; further forward the three or four nerve roots may leave the skull through two distinct openings or may be collected into a single foramen. In cases where only one anterior foramen is present, this foramen sometimes shows very clear evidence of its composite character in the fact that its extreme nerve roots, either anterior or posterior, run for some distance either in an open groove on the surface of the cartilage, or even in a closed canal in its mass, prior to entering the actual foramen. The variation in the number of the hypoglossus foramina in different stages is in no way related, in *Eumeces*, to the age of the embryo. The unquestionable bilateral asymmetry in certain embryos is striking. Apparently the variations find their chief significance as a conspicuous warning of the danger of general conclusions based upon single specimens. They may also be interpreted as an evidence of a generally distributed ability to form cartilage in situ in any part of the 'membranous skull,' as maintained by Gaupp ('93, '00), rather than by outgrowth from a comparatively few definitely localized centers.

The following tabulation illustrates the range of variation observed in the Reptilia. Apparent contradictions are doubtless due in most cases to specific differences or to individual variations, either dependent upon age or seemingly accidental as in *Eumeces*. The noticeable tendency of the earlier authors to record the smaller numbers suggests that faulty material and less exact methods have contributed to the confusion. Several of the records, notably of Siebenrock, are based upon figures, without confirmatory statement in the text. Bilateral asym-

metry is seemingly not uncommon. The presence of a number of nerve roots in excess of the number of foramina is recorded by several authors for a variety of forms; the maximum is five in *Sphenodon*, according to Schauinsland ('00) and Howes and Swinnerton ('01).

LACERTILIA

<i>Ascalabotes</i>	3	Sewertzoff, 1897.
<i>Chamaeleo</i>	1	J. G. Fischer, 1852.
<i>Eumeces</i>	2	Rice, 1911.
	3	Rice, 1911.
<i>Iguana</i>	2	J. G. Fischer, 1852.
<i>Istiurus</i>	1	J. G. Fischer, 1852.
<i>Lacerta</i>	2	J. G. Fischer, 1852; Fürbringer, 1897.
	3	Fürbringer, 1897; Gaupp, 1900.
<i>Platydactylus</i>	3	J. G. Fischer, 1852; Fürbringer, 1897.
<i>Salvator</i>	2	J. G. Fischer, 1852.

OPHIDIA

<i>Python</i>	2	Fürbringer, 1897.
<i>Tropidonotus</i>	1	Parker, 1878.
	4	Chiarugi, 1889, 1890; Gaupp, 1905 b.
<i>Vipera</i>	2	Peyer, 1912.

RHYNCHOCEPHALIA

<i>Sphenodon</i>	2	Osawa, 1898; Schauinsland, 1900; Howes and Swinnerton, 1901.
	3	Fürbringer, 1897; Schauinsland, 1900; Howes and Swinnerton, 1901.
	4	Howes and Swinnerton, 1901.

CHELONIA

<i>Chelone</i>	1	Parker, 1880 (record open to question).
	2	Gaupp, 1905 b; Fuchs, 1912; Nick, 1912.
	3	Fuchs, 1912.
<i>Chelydra</i>	2	Siebenrock, 1897; Nick, 1912.
	3	Fürbringer, 1897.
<i>Chelys</i>	3	Siebenrock, 1897.
<i>Chitra</i>	3	Siebenrock, 1897.

CHELONIA—*Continued*

Cinosternum.....	2	Siebenrock, 1897.
Cyclanorbis.....	3	Siebenrock, 1897.
Cyclemys.....	2	Siebenrock, 1897.
Dermochelys.....	1	Nick, 1912.
Emys.....	1	Noack, 1907.
	2	Fürbringer, 1897; Kunkel, 1912 b.
	3	Kunkel, 1912 b.
Geoemyda.....	2	Siebenrock, 1897.
Hydraspis.....	2	Siebenrock, 1897.
Nicoria.....	2	Siebenrock, 1897.
Podocnemis.....	2	Siebenrock, 1897.
Staurotypus.....	3	Siebenrock, 1897.
Testudo.....	2	Siebenrock, 1897.
Trionyx.....	2	Siebenrock, 1897.
	3	Fürbringer, 1897.

CROCODILIA

Alligator.....	1	Parker, 1883.
	2	Fürbringer, 1897.
Crocodilus.....	1	Parker, 1883 (Record open to question).
	2	Fürbringer, 1897; Gaupp, 1905 b.
	3	Shiino, 1914.
	4	Shiino, 1914.

In the Mammalia a single foramen has been described as the rule, but the exceptions are becoming startlingly numerous. Thus two foramina have been recorded, at least in some specimens, for *Semnopithecus* (E. Fischer, '03, cited by Mead, '09), *Talpa* (Noordenbos, '05), *Lepus* (Noordenbos, '05; Voit, '09 b), *Dasyurus* (Broom, '09), *Trichosurus* (Broom, '09), and *Lagenorhynchus* (de Burlet, '14 b, '16). In man the variability is very striking and asymmetry by no means uncommon. A partial division of the single foramen has been noted by Macklin ('14) and Kernan ('16), and a complete division, on one or both sides, by Levi ('00), Weigner ('11), and Lillie ('17); occasionally one of these foramina may be further divided, partially (Lillie) or wholly (Weigner), giving a maximum of three foramina—a condition earlier described by E. Fischer ('03, cited by Mead, '09) for one side of an asymmetrical skull of *Semnopithecus pruinosus*. "From the variations in the form of the

hypoglossal canal in adult skulls" Lillie was "unable to decide whether there were three or four hypoglossal canals developmentally" (p. 144). Conditions in embryonic Eumeces seem hardly more decisive than in adult Homo.

J. G. Fischer ('52) is cited by Gaupp ('08 a) as affirming the absence of hypoglossus foramina in *Varanus*, *Crocodylus biporcatus*, *Crocodylus acutus*, and *Alligator punctulatus*, the hypoglossus roots leaving the skull in company with the vagus through the fissura metotica; in *Istiurus* an anterior root has a similar exit, although the posterior root is provided with an independent foramen. A careful examination of Fischer's paper, rather obscure in this connection, leaves me unconvinced of the accuracy of this citation except as regards the anterior root in *Istiurus* and *Varanus*. Concerning these Fischer's statement is explicit. This record, unique for the Reptilia, must be accepted with caution until confirmed on the basis of modern technical methods; on the other hand, it is of more than passing interest, because of the fact that an identical arrangement has been long recognized in adults of *Echidna* and *Ornithorhynchus*, and has been demonstrated by Gaupp ('08 a) in all embryonic stages of the former. Gaupp holds this as confined to the monotremes alone, at least among mammals—"eine Besonderheit der Monotremen;" but de Burlet ('14 a, '16) has described the same course of the hypoglossus in an embryo of *Balaenoptera* and in adult skulls of a number of other Cetacea and Sirenia. It should be noted that a separate foramen was found in other skulls of some of these species, and that some specimens (among them the *Balaenoptera* embryo) showed the exit through the fissura metotica on one side only. Owing to the scarcity of the material, it is impossible to determine which is the normal structure and which the exceptional in these orders.

Nerve VI. The foramen for the abducens nerve (fig. 1, *f.n.VI.*) is located exactly as in *Lacerta* and, apparently, all other reptiles. The nerves pass through a pair of tunnels excavated in the thickened cartilage of the crista sellaris near its lateral limits and located each immediately above the common origin of the corresponding trabecula (*trab.*) and basipterygoid process

(*pr.b-pt.*). No conspicuous variation is noted in the different stages studied except on one side in stage 6. Here the tunnel doubly penetrates the basal plate, and for a short distance the nerve (fig. 20, left side, *n.VI.*) lies in an open groove on the ventral side of the cartilage. There is no evidence that this variation is due to the more advanced age of this embryo (the tunnel on the other side is not unusually extended toward the ventral side), nor that it has any special morphological significance; much more probable is the view that it is a mere accidental or individual variation parallel to the variations already described for the hypoglossus foramina.

While there is seemingly unanimous agreement concerning the course of the abducens in the reptiles, one point in the interpretation of this course demands consideration. It is usual to interpret the passage of the nerve through the crista sellaris as its emergence from the primary cranial cavity; thus Gaupp ('11) and Voit ('09 b), both of whom contrast this exit in the reptiles with the exit in mammals through the fenestra sphenoparietalis of the lateral wall. But examination of Gaupp's figures of *Lacerta* or of figure 1 of this paper will show conclusively that, when it emerges from the tunnel in the crista sellaris, the nerve still lies dorsal to the trabecula (*trab.*) and median to the pila prootica (*pi.pr-ot.*), rudimentary in stage 5 of *Eumeces*; in other words, it is still within the primary cranial cavity, and finds its real exit through the rather indefinite connective tissue filling the fenestra metoptica of the lateral cranial wall (see description of temporal region, p. 179). These points have been correctly emphasized by Shiino ('14). This interpretation brings the course of nerve VI in the reptile into harmony with that in the mammal except that the mammal shows no perforation of the crista sellaris. Even in this respect the bridging of the abducens by a bar of cartilage from the 'processus clinoides posterior' of the basal plate to the cochlear prominence of the ear in the pig (Mead, '09) is very suggestive of conditions in the reptile, although the fenestra thus enclosed is not strictly homologous with the hypoglossus foramen of the reptile, as pointed out by Mead himself. The notch for the abducens on the lateral

side of the 'radix dorsi sellae' figured by Voit ('09 b, fig. 6) in the rabbit is perhaps even more closely homologous with the reptilian foramen. The further course of the nerve in this figure, between the rudiments ('Restknorpel') of the primary cranial wall, emphasizes still further the general homology of mammal and reptile as regards the relations of the sixth cranial nerve.

4. OCCIPITAL REGION

The general form of the occipital region (fig. 6) is essentially as in *Lacerta*—an approximately pentagonal frame around the foramen magnum (*f.mag.*). The base of the pentagon consists of the posterior part of the basal plate, already described. From each of its posterolateral angles there arises a somewhat flattened occipital arch or pila occipitalis (*oc.*), which extends upward and outward (laterad) to the dorsolateral angle of the pentagon. Here the arch broadens suddenly and unites with the otic capsule along the median surface of the prominence of the posterior semicircular canal (*prom.s-c.p.*), thus dorsally closing the fissura metotica (*fs.m-ot.*). The expanded arch, still fused with the otic capsule, then extends dorsad and mesad into the scanty tectum posterius (*tect.p.*), which forms the apex of the pentagon. The tectum posterius is the only cartilaginous roof of the skull posterior to the nasal capsule. From the middle of the tectum a very long processus ascendens (*pr.asc.*) extends upward and forward, as in *Lacerta*, furnishing protection to the endolymph sacs. The pentagon of the foramen magnum is less regular in *Eumeces* than in *Lacerta*; its dorsolateral sides are complicated by thin projections of cartilage into the opening, and the ventral side is interrupted by the more conspicuous condylar projections and intercondylar notch of the basal plate.

In *Lacerta* the fissura metotica is carried much further dorsally than in *Eumeces*, although the upper extension is reduced to minimum width and contains no important organs; the extreme is seen in *Emys* (Kunkel, '12 b), where the occipital arches remain entirely free from the otic capsules. Complete dorsal fusion from a little below the dorsolateral angle of the foramen

magnum is the rule in all stages of *Eumeces*, as also in the crocodile (Shiino, '14). The fissura metotica will be further discussed in connection with the otic region (p. 149).

The roof of the skull in this region was originally named 'tectum synoticum' by Gaupp ('93) and 'tectum interoccipitale' by Platt ('97); later Gaupp ('06) substituted the name 'tectum posterius,' as leaving open the question of origin and morphological relation. The indifferent name is to be preferred. In stage 5 of *Eumeces* a deep groove indicates the boundary between occipital arch and otic capsule for some distance above their union, as also in *Lacerta*. By a study of the sections it is easy to follow this boundary still further because of the different histological character of the cartilage—similar to the superficial layer immediately under the perichondrium. Finally, however, the two masses of cartilage become absolutely confluent and homogeneous. Both otic capsule and occipital arch may, then, be traced into the tectum without any interruption. In her model of stage 2, Miss Cole has shown a deep dorsal notch between the tectum on the one hand and the otic capsule on the other; the notch is not continuous with the fissura metotica, although lying in the line of its extension. In this stage more than in stage 5 the conditions indicate an original development of the tectum in connection with the occipital arches and a later fusion with the otic capsules. From a careful study of the sections, I think this interpretation probable, although the undeveloped condition of the cartilage and the indefiniteness of the limits of cartilage and connective tissue make an exact determination very difficult. In stage 1 the tectum is barely suggested, and no light is thrown upon its origin.

A survey of the literature shows the utmost divergence of opinion as to the origin and relations of the tectum posterius in the reptiles. For the turtles its otic character seems to be demonstrated beyond question through the work of Gaupp ('05 b), Kunkel ('12 b), and Nick ('12), who show the occipital arches with a free dorsal termination and with no connection with the well-developed tectum. Gaupp ('05 b) holds also to its otic character in the crocodile, as does Schauinsland ('00) for *Sphen-*

odon; on the other hand, Gaupp considers that the tectum is partly occipital in *Tropidonotus*—a view confirmed by Peyer ('12) for *Vipera*. In *Lacerta*, Gaupp ('00) assigns a mainly otic character to the tectum, although not explicitly denying the participation of the occipital region in its formation.

Two suggestions have been advanced concerning the tectum of the Mammalia, where somewhat similar confusion is in evidence, either of which may contribute also to the solution of the problem for the Reptilia. First, Bolk ('03) holds for *Homo*, and Noordensbos ('05) for *Talpa*, *Lepus*, *Sus*, and *Bos* that the tectum posterius first develops as an independent cartilage and only secondarily fuses with neighboring parts. A similar independent origin has been pointed out among the Amphibia in *Rana* and *Triton* by Gaupp ('93) and in *Necturus* by Platt ('97). An independent origin has been suggested as a possibility in *Sphenodon* by Schaudinsland ('00), but I know of no definite record of this phenomenon in any reptile. Second, Kernan ('16), on the basis of his own work and that of Macklin ('14) on human embryos, holds that two distinct structures have been confused—a more anterior 'tectum synoticum' and a more posterior 'tectum posterius.' The former is more primitive and of earlier ontogenetic appearance; it is already degenerating as the latter is developing, thus leading to a gradual shifting of the position of the tectum toward the posterior, accompanied by an also gradual change in its character. A similar view is expressed by Terry ('17), who homologizes the tectum of the lizard with the anterior element. These suggestions deserve careful review in connection with the reptile skull, and may help toward a satisfactory general interpretation of the tectum posterius. As yet this is impossible.

No new light is shed by my *Eumeces* material upon the vexed question of the vertebral composition of the occipital region. The cartilages give no visible evidence of such segmentation beyond the presence of the hypoglossus foramina; the irregularities in the number of these foramina have been discussed in connection with the basal plate.

5. OTIC REGION

1. *General description*

The otic region of the skull consists primarily of the large and complicated auditory capsules at the sides, connected ventrally by the forward portion of the basal plate and dorsally, at least as regards space relations, by the tectum posterius. The question whether the tectum belongs morphologically to this region has been discussed. The whole thus forms a transverse ring of the skull, but a ring of very different dimensions in its different parts; the anteroposterior extent of the tectum posterius is very slight, while that of the otic portion of the basal plate is relatively enormous. Dorsally and ventrally the otic and occipital regions are confluent; laterally the auditory capsule is separated from the occipital arch by a narrow slit, the fissura metotica (fig. 6, *fis.m-ot.*). Anteriorly the boundary of the otic region is formed, in its median ventral portion, by the transverse ridge of the crista sellaris, to the side by the free margin of the otic capsule. From the lateral extremity of the crista to the capsule the boundary is completed by the recurved anterolateral margin of the basal plate. The complexity of the line of junction of basal plate and otic capsule, together with the peculiar position of the facialis foramen, is best postponed to a later paragraph (p. 144).

Only two pairs of cartilaginous bars connect the otic region with the anterior parts of the skull—the taeniae marginales (fig. 1, *t.marg.*), springing from the topmost points of the otic capsules, and the trabeculae baseos cranii (fig. 2, *trab.*), arising at the lateral extremities of the crista sellaris. In close proximity to the anterodorsal curve of the otic capsule, but without contact, is the upper end of the epipterygoid (fig. 3, *epipt.*). All of these structures belong rather to the temporal region and will be discussed in that connection (pp. 169 and 172).

In addition to this general outline of the otic region, certain points demand detailed discussion. These are especially the external form of the auditory capsules, the cavity of the capsule,

the foramina of the otic region, and the columella auris, which may be conveniently discussed in connection with this region, whatever its real homologies.

2. *Exterior of auditory capsule*

As already noted, the cochlear portion of the auditory capsule is relatively much larger in Eumeces than in Lacerta. As a consequence, the general form of the capsule is strikingly different in the two animals, as seen from figure 32, in which the capsules of Eumeces (solid lines) and Lacerta (broken lines) are combined. The outline for Lacerta is derived from Gaupp's figure 7, that for Eumeces from figure 8 of this paper. In Lacerta the general form is a horizontal oval, with the larger end to the posterior and the long axis extending from posteromedial to anterolateral; the ventral contour of the oval is somewhat irregular because of the protrusion of the cochlear prominence. On the other hand, in Eumeces the cochlear prominence is so exaggerated as to give to the entire capsule a pear-shaped outline (fig. 7), in which the slender stem end (cochlear prominence), extending downward and forward, determines a new long axis. While the long axes of the two capsules in Lacerta and the corresponding axes of their upper utriculosaccular portions in Eumeces diverge anteriorly (fig. 1), the new long axes in Eumeces are almost exactly parallel to the sagittal plane of the head. A further relative increase in the size of the cochlear portions of the capsules might well lead to the anterior convergence characteristic of the Mammalia. As seen from the front, the capsule of Eumeces has rather the form of a half pear, the median contour being approximately plane, while the lateral contour, especially in the upper utriculosaccular portion, is conspicuously convex.

Aside from the contrast in form resulting from unequal development of the cochlear prominence, the superficial topography of the otic capsule agrees very closely in Eumeces and Lacerta. On the lateral surface (fig. 7) the prominences corresponding to all three of the semicircular canals are recognizable, the anterior

(*prom.s-c.a.*) being most sharply marked and forming the entire anterodorsal border of the rather triangular upper portion of the capsule. This prominence is marked off from the rest of the capsule by a sharp groove, plainest in its middle course and becoming less definite at the anterior end, where the prominence of the canal broadens out into that of its ampulla (*prom.amp.a.*). At the upper angle of the capsule a slight notch separates the prominence of the anterior from that of the posterior semicircular canal (*prom.s-c.p.*), which is much less clearly marked than the anterior, being limited by a very shallow and rather indefinite depression. The prominence of the posterior canal broadens at its lower end, forming a conspicuous projection, the prominence of the posterior ampulla (*prom.amp.p.*). By the contact of this prominence with the edge of the basal plate the fissura metotica is divided, as already noted, into its anterior and posterior parts. Posterior and dorsal to the prominence of the anterior ampulla is that of the lateral ampulla (*prom.amp.l.*), and from this the prominence of the lateral canal (*prom.s-c.l.*) extends backward, forming the main lateral convexity of the auditory capsule. Posteriorly it merges with the prominence of the posterior canal above the posterior ampulla. For the most part the prominence of the lateral canal is poorly marked, but in its posterior third the cartilage is much thickened and forms a conspicuous projection, the crista parotica (*cr.par.*), discussed later in connection with the columella auris (p. 155). Immediately below the crista, at the beginning of the elongate cochlear prominence (*prom.coch.*), is the large elliptical fenestra vestibuli (*fen.vest.*), partially filled by the footplate of the columella auris (*ft.pl.*). The *prominentia saccularis* (*prom.sac.*), bounded by the semicircular canals, and the *prominentia recessus utriculi* (*prom.r.ut.*), ventral and posterior to the anterior ampullar prominence, are as in *Lacerta*.

In median view (fig. 8) the prominences of the anterior semicircular canal, anterior ampulla, lateral ampulla, posterior ampulla, and cochlea are again recognizable, although the contours are in general less sharply marked than on the outer face and less sharply marked than in *Lacerta*. The middle of this surface of

the ear capsule is slightly swollen to form the *prominentia utricularis* (*prom.ut.*), dorsoventrally constricted in front, where, as *prominentia recessus utriculi* (*prom.r.ut.*), it extends under the prominences of the anterior and lateral ampullae to reach the margin of the auditory capsule and unite with the basal plate. In its posterior extent the utricular prominence is separated from the prominence of the anterior canal by the conspicuous fossa subarcuata (*fos.s-a.*), and perforated by the long slit-shaped foramen endolymphaticum (*f.end.*). In the line connecting the lower end of the endolymphatic foramen with the facialis foramen, and slightly nearer to the former, is the foramen acusticum posterius (*f.n.VIII.p.*), opening into the *cavum vestibulare posterius* (fig. 5, *cav.vest.p.*); the foramen acusticum anterius (*f.n.VIII.a.*), again, lies midway between the posterior acusticus foramen and the facialis foramen, but distinctly lateral and dorsal to the line connecting the two. This anterior acusticus foramen opens upward and forward into the *cavum vestibulare anterius* (fig. 5, *cav.vest.a.*), and is arched over by a projecting lip of cartilage. On the ventroposterior surface of the *prominentia cochlearis*, and visible in neither lateral nor median view, is the large fenestra cochleae (fig. 2, *fen.coch.*; in figs. 7 and 8 its position is indicated by an arrow), opening from the cochlear cavity into the *fissura metotica*.

In other reptiles, also, the otic capsule shows only minor variations. An enlargement of the cochlear portion, similar to that of *Eumeces*, is described in the crocodile (Gaupp, '05 b, with reproduction of figure from Parker, '83; Shiino, '14). The latter author notes also a sharp bend toward the median line in the course of the cochlear prominence. On the other hand, the cochlear prominence in the turtles, according to Kunkel ('12 b) and Nick ('12), is less developed than in *Lacerta*, reaching the minimum in *Chelone* (Gaupp, '05 b). In *Emys* (Kunkel, '11, '12 b) the cochlea is described as extending to the posterior; in all other forms it extends to the anterior. In *Crocodylus* (Shiino, '14) and the earlier stages of *Vipera* (Peyer, '12) the entire capsule is so rotated that the anterior semicircular canal lies

in an almost horizontal position along the dorsal margin, while the lateral 'horizontal' canal is nearly vertical. In the turtles Nick and Kunkel report the exterior modeling as much less delicate than in the lizards.

3. Interior of auditory capsule

In the study of the interior of the ear capsule I make free use of an unpublished thesis by Mr. Joseph I. Taggart. The interpretation of the space relations of a cavity is notoriously difficult, even when the cavity is far less complicated than that of the ear. With this difficulty in view, Taggart made a solid model or cast of the cavity of the otic capsule of stage 5 of *Eumeces*, reproduced in figure 5 of this paper. The membranous labyrinth was also modeled for comparison. As there are no striking departures from *Lacerta*, a brief summary will suffice in connection with the figure.

The principal division of the cavity is the *cavum vestibulare posterius* (*cav.vest.p.*), with its three extensions—anteroventral, the *cavum cochleare* (*cav.coch.*); posteroventral, the *recessus ampullaris posterior* (*r.amp.p.*); posterodorsal, the *recessus pro sinu superiore utriculi*, located below the junction of the anterior and posterior semicircular canals (*can.s-c.a.* and *can.s-c.p.*), but invisible in the figure because of its position median to the *cavum vestibulare posterius*. To the anterior the *cavum vestibulare posterius* is connected, but less intimately, with the *cavum vestibulare anterius* (*cav.vest.a.*). The courses of the three semicircular canals are easily followed in the figure.

In the lateral part of the *cavum vestibulare posterius* is located the *sacculus*, with which are connected the *cochlea* and the *ductus endolymphaticus*. The *cochlea* extends downward and forward into the *cavum cochleare*, while the *ductus endolymphaticus* passes to the median wall of the *cavum* and through the *foramen endolymphaticum* into the brain cavity, where it expands into the capacious *endolymph sac*. Median to the *sacculus* in the *cavum vestibulare posterius* is a part of the *utriculus*, enlarged

posteriorly, slender and tube-like to the anterior, where it passes dorsal to the endolymph duct and extends forward into the *cavum vestibulare anterius*. Here it expands again to form the *recessus utriculi*. The posterior enlargement of the *utriculus* connects with the anterior and posterior semicircular canals by way of the *sinus superior*, lying in the *recessus* of the same name, and separately with the posterior limb of the lateral canal; it has also a connection with the posterior ampulla, which occupies the *recessus ampullaris posterior*. While the form of the *cavum vestibulare posterius* gives little suggestion of its complex contents, the *cavum vestibulare anterius* is far more expressive. As seen in lateral view, it is rather definitely triangular and noticeably inflated in the neighborhood of each angle. The posteroventral angle contains the *recessus utriculi*, already mentioned, while the anterior and lateral ampullae lie in the anterior and postero-dorsal angles, respectively.

In the cast (fig. 5) the *fenestra vestibuli* (*fen.vest.*), *fenestra cochleae* (*fen.coch.*), and anterior acoustic foramen (*f.n.VIII.a.*) are represented by projecting plugs, and the impressions of neighboring cartilages appear as hollows in the plugs—the foot-plate of the *columella auris* in that of the *fenestra vestibuli* and the edge of the basal plate in that of the *fenestra cochleae*. The *foramen endolymphaticum* and the posterior acoustic foramen, on the median side of the *cavum vestibulare posterius* and of the transition from this *cavum* to the *cavum cochleare*, respectively, are not seen in the figure.

The thin plates of cartilage separating the semicircular canals from the *cava vestibularia*, *anterius* and *posterius*, are named by Gaupp the *septa semicircularia*—*anterius*, *laterale*, and *posterius* respectively. The transverse plate between the two *cava vestibularia* is known as the *septum intervestibulare*; the foramen through which the two *cava* communicate is the *foramen intervestibulare*. In the turtles, *Emys* (Kunkel, '12 b), *Dermochelys*, *Chelone*, and *Chelydra* (Nick, '12), the *septum intervestibulare* is wanting, and the anterior and posterior chambers unite in a common *cavum vestibulare*; in *Chelydra* Nick also

describes the septa semicircularia as very vestigial, so that cartilaginous semicircular 'canals' can scarcely be said to exist. *Vipera* (Peyer, '12) is seemingly essentially like *Lacerta* and *Eumeces*.

4. *Foramina of otic region*

In the otic region and on its borders are a number of nerve foramina and other openings which may well be considered together—fenestra prootica, facialis foramen, anterior and posterior acoustic foramina, foramen endolymphaticum, fenestra vestibuli, fenestra cochleae, and fissura metotica. In addition may be mentioned certain 'holes' ('Lücken' of Gaupp) due merely to retarded chondrification. Most of these openings have been mentioned above, but several require more extended discussion and comparison.

Fenestra prootica (fig. 33, *fen.pr-ot.*). The fenestra prootica, bounding the otic capsule anteriorly, is better discussed with the temporal region (p. 179).

Facialis foramen (figs. 7 and 8, *f.n.VII.*). The changed form of the line of union of the otic capsule with the basal plate and the changed position of the facialis foramen, as compared with *Lacerta*, have been mentioned briefly, but must be considered further. In both forms the otic capsule and basal plate are continuously fused from the fissura metotica to the fenestra prootica, save for the interruption by the facialis foramen, which divides a 'prefacial' from a 'postfacial basicapsular commissure' (Gaupp). The prefacial connection in *Eumeces* differs in no essential point from that of *Lacerta*—a simple line of junction of the basal plate with the otic capsule (mainly the prominentia recessus utriculi) anterior to the acoustic foramina, dorsolateral rather than anterior with reference to the facialis foramen itself. The entire line of contact of otic capsule and basal plate from the facialis foramen to the fissura metotica must be interpreted as belonging to the postfacial commissure of Gaupp; but here *Lacerta* and *Eumeces* differ startlingly. Gaupp ('00, p. 446) writes of *Lacerta*: "Längs der ausgedehnten postfacialen basikapsulären Ver-

bindungslinie geht die Basalplatte kontinuierlich in den vorderen und in den medial-ventralen Umfang der Pars cochlearis über." In Eumeces the cochlear portion of the ear capsule has been so elongated in an anteromedian direction that the line of the postfacial connection has become deeply U-shaped, with laterodorsal and medioventral limbs and an anterior loop; the facialis foramen has come to lie strongly lateral and dorsal rather than anterior to the cochlear prominence. In Lacerta a line from the facialis foramen to the anterior end of the fissura metotica hardly more than touches the cochlear prominence; a similar line in Eumeces cuts off a considerable portion. The contrast is shown in figure 32, in which the broken lines represent Lacerta and the solid lines Eumeces. The drawing of Lacerta is based upon figure 7 of Gaupp; his figure 1 would make the contrast even more striking. In Eumeces the 'postfacial' connection has become rather infrafacial (in part even prefacial), although it is probably convenient to retain Gaupp's terminology.

The similarity of the position of the facialis foramen in Eumeces and the mammals is striking—in a deep notch of the otic capsule, between the cochlear prominence and the anterior dome of the utricular portion, containing the recessus utriculi and the anterior and lateral ampullae. Gaupp ('00, pp. 509, 510) gives the following interesting comparison:

Bei den Amphibien liegt es (i.e., the facialis foramen), die mannigfachen oben zum Teil erwähnten Besonderheiten beiseite gelassen, auf der Grenze zwischen der 'Ohrkapsel' und der soliden 'Basalplatte;' bei den Sauriern finden wir den vordersten Teil des Abschnittes der Ohrkapsel, der die Cochlea beherbergt, schon ventral von dem Facialisloch; bei den Säugern liegt das Facialisloch an der dorsalen Kante der Ohrkapsel; nicht nur ventral, sondern auch vor ihm findet sich ein Abschnitt der Ohrkapsel. . . . In der Skelettbrücke, die bei den Säugern . . . den ersten, ältesten Abschnitt des Facialiskanals (bis zum Hiatus spurius) dorsal abschliesst und die den vorderen cochlearen Teil der Ohrkapsel mit dem hinteren verbindet, sehe ich somit die der präfacialen Kommissur der niederen Vertebraten entsprechende Skelettbrücke. Dass man sie bei den Säugern nicht mehr basikapsulär, sondern interkapsulär nennen kann, hat eben seinen Grund darin, dass ein Teil des Craniums, der bei den Sauriern noch ungeteilt war, 'Basalplatte' bildete, bei den Säugern Cochlearteil der Ohrkapsel geworden ist.

Except for the changes in the orientation of the capsule in the mammals, due to the reduction in the relative size of the semi-circular canals, *Eumeces* conforms more closely to the above description of the mammal than to that of the reptile, even to the presence of the intercapsular plate of cartilage bounding the facialis foramen anteriorly. Attention should, however, be called to the limitation of the homology with the reptilian foramen faciale to the central part only of the mammalian canalis facialis; the distal portion of this canal, beginning at the hiatus spurius and the facialis ganglion, is unquestionably a secondary addition.

In a slightly different connection, Voit ('09 a) has given a series of very interesting diagrammatic figures illustrating the comparative relations of the facialis foramen, nerve, and ganglion in reptiles and mammals. Except for the newly added lateral wall of the secondarily enlarged cavum cranii, his figures for the mammal (figs. 1 c and 1 c₁) might almost have been drawn from my sections of *Eumeces*. Noordenbos ('05) has also emphasized the essential homology of the facialis foramen in mammal and reptile, but describes the mammalian foramen as wholly surrounded by the cartilage of the otic capsule. Gaupp's interpretation of the intercapsular bridge in the mammals as belonging to the basal plate seems far more probable. It is unquestionably such in *Eumeces*.

In the passage just quoted, Gaupp has explained the difference between mammal and reptile as due to the progressive invasion of the basal plate by the enlarging cochlea—an actual appropriation, for the formation of the cochlear capsule in the higher forms, of material serving as part of the basal plate in the lower forms. This process begins in the reptiles and culminates in the mammals. This view, reiterated in later papers ('05 b, '06, '08 a), has met with rather general acceptance. Noordenbos ('05), however, has criticised it severely, as applied to the Mammalia, on the ground of the independent ontogenetic development of the otic capsule and its relatively late fusion with the basal plate, and Schauinsland ('00) apparently describes a similar inde-

pendent development in Sphenodon. On the other hand, Gaupp ('08 a) finds conditions in *Echidna* in full accord with his theory. Terry ('17) harmonizes the views of Gaupp and Noordenbos. Describing the otic capsule of the cat as chondrifying independently of the main part of the basal plate, but interpreting the suprafacial commissure, after Gaupp, as a parietal portion of the basal plate, he concludes (p. 363):

If we can, on the evidence given, interpret the suprafacial commissure as a parietal structure in the cat, it would appear that its relation to the cochlear capsule (continuity) affords support to the theory (Gaupp) of the latter having preempted the territory of the basal plate and developed at its expense.

The evidence of *Eumeces*, while not conclusive, is favorable to Gaupp's view, and adds one more to the series of gradations afforded by the *Reptilia* intermediate to the extreme conditions found in *Amphibia* and *Mammalia*. In *Chelone* (Gaupp, '05 b; Nick, '12) and *Dermochelys* (Nick, '12) the invasion has hardly begun and conditions are still almost as in the *Amphibia*; *Chelydra* (Nick, '12) and *Emys* (Kunkel, '12 b) lead up to *Lacerta*; *Crocodylus* (Gaupp, '05 b, citing and confirming Parker, '83; Shiino, '14) and *Eumeces* show the maximum of this development in the reptiles. From the mammalian side the gap is further decreased by the observation that in *Echidna* (Gaupp, '08 a) and marsupial embryos (Broom, '09) the uncoiled cochlea and its relations to the basal plate are still extraordinarily reptilian.

Foramina acustica, anterieus and posterius (fig. 8, *f.n.VIII.a.* and *f.n.VIII.p.*). In stages 5 and 6 of *Eumeces* these foramina are essentially as in *Lacerta*. As in *Lacerta*, the anterior foramen is so overhung by a lip-shaped projection of cartilage that the opening itself is barely visible in median view. In earlier stages the cartilage between the acusticus foramina is less definite, and in stage 1 these and the endolymphatic foramen become perfectly confluent—a condition already described by several authors in a variety of reptiles and mammals. In no stage do I find any suggestion of the division of the anterior foramen into

two as described in *Chelydra* by Nick ('12, citing and confirming Siebenrock, '97).

Foramen endolymphaticum and 'holes' due to retarded chondrification (fig. 8, *f.end.*). In stage 5 of *Eumeces* the foramen endolymphaticum is remarkably elongate in a direction approximately parallel to the fissura metotica. The ductus endolymphaticus leaves the ear capsule through the anteroventral end of the foramen, the posterior portion of the slit being filled out with a confused mass of procartilage and connective tissue which makes the exact determination of boundaries extremely difficult. Owing to this fact, the size of the aperture may well have been exaggerated in the modeling; in a second model from the same sections (fig. 1) the foramen was represented as somewhat shorter, and, on one side, as divided by a bar of rather questionable cartilage. But, after all allowances, the large size and elongate form of the foramen endolymphaticum in this and earlier stages are beyond question. A comparison with stage 6 shows that the condition figured is only temporary, the foramen in the later series being circular or nearly so, and only large enough to permit the passage of the slender duct. The major part of the slit in the earlier stages is due to a mere retardation of the cartilage development, and its later filling in with cartilage is to be interpreted as the further progress of the process, described in the preceding paragraph, by which the foramen endolymphaticum was originally separated from the acusticus foramina. An exactly similar change in the form and size of this foramen is described in the pig by Mead ('09). In *Chelone* and *Dermochelys*, but not in *Chelydra*, Nick ('12) reports a degeneration of the saccus endolymphaticus in later stages, accompanied by the complete obliteration of the foramen endolymphaticum.

A similar delay in chondrification is responsible for the hole ('Lücke') described by Gaupp in the cochlear prominence of *Lacerta*. Other such holes have been reported in various parts of the otic capsule by Kunkel ('12 b) in some specimens of *Emys*, by Nick ('12) on one side in a single specimen of *Chelydra*, and by Peyer ('12) in *Vipera*. In stage 5 of *Eumeces* similar minute

interruptions of the cartilage are apparently present in the bottom of the fossa subarcuata (fig. 1). The character of the filling tissue is very problematical; it was first interpreted as cartilage, later as procartilage. Hence the difference in figures 1 and 8, drawn from two different models based upon the same series of sections. On one side there are two such openings, leading, respectively, into the cavum vestibulare anterius and cavum vestibulare posterius; on the other side the opening into the cavum anterius cannot be recognized. The filling of all three in stage 6 is unquestionably cartilaginous.

Fenestra vestibuli (fig. 7, *fen.vest.*). The fenestra vestibuli is decidedly elongated parallel to the prominentia cochlearis (dorso-posterior to ventroanterior), and slightly larger, at least in stages 5 and 6, than the footplate of the columella auris which rests in it. In *Lacerta* the fenestra is circular; in *Emys* (Kunkel, '12 b) it is triangular. In earlier stages of *Eumeces* the cartilages of footplate and cochlear wall are seemingly continuous, thus obliterating any distinct fenestra vestibuli (see also p. 163). The position with reference to the entire otic capsule is essentially the same in *Eumeces* and *Lacerta*, although the enlargement of the cochlea gives it the appearance of being much more dorsal in *Eumeces*.

Fenestra cochleae and fissura metotica (figs. 2, 7, and 8, *fen.coch.* and *fis.m-ot.*). The relation of the fenestra cochleae to the fissura metotica is so intimate that the two may be advantageously discussed together. As already noted, the fissura metotica is terminated dorsoposteriorly much sooner in *Eumeces* than in *Lacerta* by a sudden flaring of the occipital arch and its union with the prominence of the posterior semicircular canal. The examination and modeling of earlier and later stages of *Eumeces*, stages 2 and 6, fully confirm this fusion. In *Lacerta* the dorsal extension of the fissure is without essential significance, being filled with connective tissue and containing neither nerves nor important blood-vessels. Gaupp ('00, p. 445) also describes an individual variation in which the fissure is almost completely obliterated above the dorsolateral angle of the foramen magnum,

although opening slightly again at its extreme dorsal limit. This approaches closely the conditions in *Eumeces*.

The fissura is divided sharply into an anteroventral recessus scalae tympani (fig. 8, *r.sc.ty.*) and a posterior foramen jugulare (fig. 8, *f.jug.*). In *Lacerta* the incomplete separation is accomplished by a mere narrowing of the fissure and a rather denser filling of connective tissue. In *Eumeces* the posterior ampullar prominence, just dorsoposterior to the fenestra cochleae, and the basal plate, in the region of the anterior hypoglossus foramen, are in very close apposition (the histological structure indicates that there is no absolute fusion of the cartilages), thus completely dividing the two parts. In stage 2 the contact is less certainly distinguished, owing to the embryonic nature of the tissues; in stages 4 and 6 the conditions are clearly as in stage 5.

The foramen jugulare furnishes the exit for the confused mass of roots of nerves X and XI, and for a large vein just posterior to the nerves. This vein is homologized by Gaupp with the mammalian jugular; I have not followed its relations sufficiently to estimate his interpretation, but can confirm his observations.

Conditions in the recessus scalae tympani are of especial interest and demand fuller consideration. Stage 5 of *Eumeces* shows a very close similarity to *Lacerta*. Figure 12 represents a section corresponding to Gaupp's figure 17. In the triangle whose apices are marked by the edge of the basal plate and the median and lateral rims of the fenestra cochleae is the very loose connective tissue in which the saccus perilymphaticus will develop, while the median and lateral apertures of the recessus (*r.sc.ty.m.* and *r.sc.ty.l.*) are closed by a much denser tissue, forming rather definite membranes. A few sections further back (fig. 11) the median aperture disappears through the approximation of the cartilages of otic capsule and basal plate. The membrane closing the lateral aperture is less developed in this region. The membrane closing the lateral aperture of the recessus Gaupp compares with the secondary tympanic membrane of the mammal, but he insists that the similarity is physiological rather than morphological, and that there can be no close homology

because the membrane in *Lacerta* is developed in an entirely different position ("an ganz anderer Stelle") from that in the mammal. While the mammalian membrane is attached wholly to the capsular rim of the fenestra cochleae, that of *Lacerta* is stretched from the rim of the fenestra to the edge of the basal plate.

In stage 6 of *Eumeces* the conditions are so modified as to suggest a slightly different interpretation and a closer homology with the mammals. Here (fig. 15) there is a less definite connection of the closing membrane with the basal plate—more of a tendency to close the entire fenestra cochleae with a single slightly convex sweep of membrane, only loosely connected with the basal plate. In the region of cartilaginous closure of the median aperture (fig. 13) the membrane is highly developed in this stage. It should also be noted that this membrane conforms very closely to the general contour of the ear capsule, so that the developing saccus perilymphaticus is almost as strictly intracapsular in this stage of *Eumeces* as in the mammal. It will be readily seen that this interpretation greatly restricts the extent of the recessus scalae tympani as an open space of the fissura metotica, external to the otic capsule; for, in the entire region of the fenestra cochleae, the otic capsule (formed here of connective tissue instead of cartilage) and the basal plate must be considered as practically in contact. In front of the fenestra cochleae the fissura metotica expands again to a considerable cavity, filled only with a rather loose and undifferentiated connective tissue and containing neither nerves nor important vessels.

The above considerations indicate that the homology of mammal and reptile in this point is closer than was held by Gaupp in his paper of 1900, but the argument may be carried a step further. In the same paper Gaupp advanced the hypothesis that a primitive fenestra cochleae of the mammal, exactly equivalent to that of the reptile, secondarily divides to form the definitive fenestra cochleae (foramen rotundum) and the aquaeductus cochleae. This surmise has been brilliantly confirmed by his own work on *Echidna* ('08 a), in which the primitive reptilian character is

retained, and by that of E. Fischer ('01 b, 1903) on *Talpa* and *Semnopithecus*, Voit ('09 b) on the rabbit, Olmstead ('11) on the dog, Macklin ('14) and Kernan ('16) on human embryos, and Terry ('17) on the cat, in all of which forms transitional stages were observed. In view of these discoveries, I believe that the lateral part of the membrane filling the fenestra cochleae of stage 6 of *Eumeces* (corresponding to the filling of the lateral aperture of the recessus scalae tympani in *Lacerta* and stage 5 of *Eumeces*) may be safely homologized with the secondary tympanic membrane of the mammal, while the median portion (corresponding to the filling of the median aperture) occupies the position of the aquaeductus cochleae of the mammal. I have been unable to demonstrate an actual connection here between the lymph spaces of the developing saccus perilymphaticus and those of the cranial cavity, but such may well appear in later stages.

Conditions in the region of the recessus scalae tympani are decidedly more complicated in the turtles (Gaupp, '05 b; Nick, '12; Kunkel, '12 b) than in the lizards. This is due in part to the different course of the glossopharyngeal nerve (described in the next paragraph) and in part to the presence of the problematical 'ductus hypoperilymphaticus' described by Nick and Kunkel. Versluys ('98) has emphatically denied the homology of the fenestra cochleae or rotunda of the reptile with the foramen rotundum of the mammal. His argument has been considered somewhat fully by Gaupp ('00). In part, at least, the recognition for the mammal of the division of a primitive fenestra cochleae into definitive foramen rotundum and aquaeductus cochleae would remove the ground of difference.

In this connection it is well to consider certain apparent anomalies in the course of the glossopharyngeal nerve in different reptilian forms. In the lizards (Versluys, '98; Gaupp, '00), *Sphenodon* (Schauinsland, '00), and crocodiles (Parker, '83, cited and confirmed by Gaupp, '05 b and '11; Shiino, '14) the course is 'extracapsular'—i.e., the nerve leaves the skull through the fissura metotica, between the otic capsule and the basal plate.

This course is also recorded as characteristic of Amphibia (Gaupp, '11) and Mammalia (Fuchs, '10; Gaupp, '11). On the other hand, in turtles the nerve is described by Gaupp ('05 b), Noack ('07), Fuchs ('10), Nick ('12), and Kunkel ('12b) as having an 'intra-capsular' course—entering the otic capsule through an independent 'foramen glossopharyngei internum' in its median cartilaginous wall, and emerging through a similar independent 'foramen glossopharyngei externum' in its lateral wall. This course is also apparently characteristic of the snakes, although the records for this group are not above reproach. Gaupp ('11) mentions this course as probable in *Tropidonotus*. Möller ('05) describes the internal foramen for all stages of *Vipera*, but records a curious variability for the exit from the otic capsule—sometimes through an independent foramen, sometimes through the fenestra cochleae and sometimes through the fenestra vestibuli. Peyer ('12), also working on *Vipera*, describes the glossopharyngeus as emerging through the fissura metotica, but mentions another undetermined nerve as penetrating the wall of the otic capsule. Is it possible that this undetermined nerve is really the glossopharyngeus, and the so-called 'glossopharyngeus' a part of the vago-accessorius complex?

Gaupp has given a very detailed description of the extra-capsular course of the nerve in *Lacerta*, with which stage 5 of *Eumeces* is in the fullest agreement. In figure 12 the nerve (*n.IX.*) is seen passing from the cavum cranii through the membrane closing the median aperture of the recessus scalae tympani; in figure 10 it lies within the recessus; in figure 11 it is emerging through the closing membrane of the lateral aperture. If the interpretation of the preceding paragraphs is correct, and the closing membrane of the fenestra cochleae (or of the median and lateral apertures of the recessus scalae tympani) is really a part of the otic capsule, then the glossopharyngeus of *Lacerta* and of stage 5 of *Eumeces* really passes through the otic capsule in a manner not essentially different from that in the turtles. Whether conditions are the same in *Sphenodon* and the crocodiles is not clear from the brief accounts available, but this is highly probable.

The examination of other stages of *Eumeces* throws further light on this point. In stage 6 the glossopharyngeus passes from the brain cavity (fig. 15, *n.IX.*) through the cartilage of the median wall of the otic capsule just dorsal to the median aperture of the recessus scalae tympani (fig. 14) into its cavity (fig. 13). Here there can be no question of the 'intracapsular' course of the nerve, although its final exit, as in stage 5 of *Eumeces* and in *Lacerta*, is through the membrane of the lateral aperture of the recessus, and not, as in the turtles, through the cartilage. In stages 2 and 3 the conditions could not be determined with certainty, but apparently stage 3 is like stage 6, while stage 2 resembles stage 5. Stage 4 is unquestionably like stage 6. The difference is, then, not one of age, but rather due to individual variability, as in the case of variations in the hypoglossus and abducens foramina. From the available material it is impossible to determine which condition (if either) is the characteristic one in *Eumeces*. This variation suggests a method by which the 'extracapsular' course of the glossopharyngeus, as seen in the *Amphibia* (Gaupp, '93, '05 b), may phylogenetically have been transformed into the typically 'intracapsular' course in the turtles through a gradual extension of the otic cartilage. Gaupp ('05 b) suggests that the change has occurred through a striking narrowing of the recessus scalae tympani; I would suggest rather a narrowing of the fenestra cochleae. In stage 6 of *Eumeces* the cartilage of the median wall has been sufficiently extended to surround an inner glossopharyngeus foramen; in the turtle a similar lateral extension has enclosed the foramen externum. In this connection it is interesting to note the very small size of the fenestra cochleae in *Emys* (Kunkel, '12 b, fig. 29). A more detailed knowledge of the later embryonic development of this region and its adult anatomy is highly desirable.

5. Crista parotica and columella auris

My study of these extraordinarily interesting and perplexing structures is by no means satisfying. I can, at least, record my observations, which are not fully in accord with those of Gaupp for *Lacerta*; my interpretations must be more or less tentative.

The crista parotica (fig. 7, *cr.par.*) has already been mentioned as a thickened ridge extending along the posterior portion of the prominence of the lateral semicircular canal and terminating in a conspicuous spur. The position is as in *Lacerta*. In Emys Kunkel ('12 b) locates the crista parotica much more posteriorly and ventrally, upon the ventral margin of the posterior ampullar prominence; its relation to the quadrate is, however, identical. An even more aberrant position is suggested by Bender ('11), who describes the crista in *Testudo* as perforated by the fenestra vestibuli. The projecting spur of cartilage makes a double bend, extending first downward and forward, then upward and forward, and finally downward and forward again. The entire structure may be interpreted as composed of distal and proximal portions, practically parallel to one another, and united by a transverse connecting piece. That the proximal portion belongs to the crista, in the restricted sense, and that the distal portion (*pr.par.*) corresponds to the processus paroticus, as distinguished by Gaupp, cannot be questioned. In passing, attention should be called to a difference in the terminology of Gaupp and Versluys ('03). The latter uses the name 'processus paroticus' as essentially synonymous with the 'crista parotica' of Gaupp; Gaupp's 'processus paroticus' he terms 'intercalare' or 'processus dorsalis' (i.e., of the columella auris). I follow the terminology of Gaupp.

In as late a stage of *Lacerta* as that furnishing the basis of his paper, Gaupp describes the crista parotica and processus paroticus as distinct cartilages, although connected by a cell-rich tissue "das in Hämatoxylin auch einen bläulichen Schimmer zeigt, aber durchaus kein hyaliner Knorpel ist." Later this connection chondrifies. In stages 4, 5, and 6 of *Eumeces* the entire structure is a unit, with no differentiation of the cartilage recog-

nizable in the different parts; in earlier stages the middle connecting portion is decidedly less advanced than the distal and proximal ends, and, in stage 2 hardly deserves the name of cartilage. Because of the direction of the plane of the sections, no further evidence upon this point can be obtained from stage 1. Beginning with stage 2, the course of development is from greater distinctness to less, and is suggestive of primary independence and secondary fusion. In any case, it is practically convenient to retain the two terms *crista parotica* and *processus paroticus*. The lateral surface of the *processus paroticus* is described by Gaupp as in contact with the quadrate. In *Eumeces* I find the quadrate and the *processus paroticus* closely united in all observed stages. In the earlier embryos there is apparently perfect confluence of the two cartilage or procartilage masses; even in my latest stages they are still in union, although a marked histological differentiation characterizes the line of junction.

Versluys ('03) traces the *processus paroticus* ('intercalare') back to an outgrowth of the *columella auris*—the *processus dorsalis*. When the *processus dorsalis* is transformed into the *processus paroticus*, its connection with the stalk of the *columella* may be retained as a band of connective tissue. This Versluys ('98) has described in adults of *Agamidae* (except *Amphibolurus*) and *Phrynosoma*, although it is usually lost. The connection is described by Gaupp in embryonic *Lacerta*. In *Eumeces* no connection between the *processus paroticus* and the *columellar stalk*, either cartilaginous or of connective tissue, could be observed in any stage. There is nothing in the conditions in *Eumeces* to suggest Versluys's interpretation; on the other hand, there is, in the purely negative evidence, nothing hostile to his view. Gaupp ('05 b) has accepted Versluys's interpretation, although retaining his own terminology.

The dorsal process, as such, is present in the crocodiles (Parker, '83, cited and confirmed by Gaupp, '05 b, and Versluys, '03; Shiino, '14) and *Sphenodon* (Versluys, '03; Fuchs, '09). In the latter it is retained in the adult; in the former Shiino notes that there is no connection with the *crista parotica*. For *Lacerta* Cords ('09) confirms the record of a *processus dorsalis* freed to

form a processus paroticus. For the turtles the data are divergent. In Emys Kunkel ('12 b) notes that no separate processus paroticus is present, and the occurrence of a processus dorsalis in Testudo is denied by Bender ('12, correcting an error in his paper of 1911, in which the foot plate of the columella was mentioned under this name). Parker ('80), however, records its presence in Chelone; and in a late stage of Chelydra Nick ('12) notes that the end of the ossified crista parotica, in contact with the quadrate, remains cartilaginous. This may perhaps be a remnant of the processus paroticus.

Versluys ('98) has given a generalized description and figure of the lizard columella auris, to which Lacerta conforms in extraordinary detail; the agreement of Eumeces is less complete. For convenience of description, the columella may be divided into three parts—a footplate, fitting into the fenestra vestibuli; an insertion plate, connected with the tympanic membrane, and a rather slender stalk, connecting the footplate and insertion plate. It is further convenient to distinguish as stapes the proximal portion of the columella, and to apply the name extracolumella to the distal portion. In adult reptiles the stapes ossifies, while the extracolumella remains cartilaginous; thus the distinction is very clear. On the other hand, the junction may be optically unrecognizable in the cartilage of the embryo, as is the case in my specimens of Eumeces. It is located in the stalk, just proximal to the processus dorsalis and processus internus described below. In the naming of processes of the columella, I follow the usage of Versluys, adopted also by Gaupp. In the following formal comparison with Lacerta, the description applies to stage 5 of Eumeces unless otherwise specified.

The fenestra vestibuli and footplate of the columella in Eumeces are not circular, as in Lacerta, but strongly elliptical, the long axis extending from posterodorsal to anteroventral. The fenestra is decidedly larger than the footplate, leaving a considerable space, especially at the ends, which is filled with non-cartilaginous tissue. Of the 'double fenestral structure' and the 'stapes inferior' described by Kunkel ('12 a) in Emys I find no suggestion. The stalk, instead of being attached at the middle

of the footplate, is attached near its lower extremity, and extends almost directly outward, so that the columellae of the two sides lie almost precisely in the same line. A little beyond its middle, the stalk carries a well-marked process (fig. 7, *pr.int.*), extending downward and very slightly forward. This is undoubtedly to be homologized with the processus internus of Versluys, although it differs in some points from the corresponding structure in *Lacerta*. In *Eumeces* the process is directed more ventrally and less anteriorly than in *Lacerta*; thus it does not reach or even closely approximate the quadrate. It is also much less strongly developed; in stage 6 it is hardly, if at all, recognizable—a condition according well with the observation of Versluys ('98) that the processus internus is lacking in adult *Scincidae*. Among the lizards Versluys ('98, '03) also records the absence of this process in adult *Uroplatae* and *Anguidae*, and in embryos and adults of the *Geckonidae*, although he cites Peters ('69) as authority for its presence in an embryonic geckonid, *Hemidactylus*. In *Sphenodon* Versluys ('03) reports the process lacking; Schauinsland ('00), on the other hand, homologizes the 'Insertionstheil (mit dem Quadratum)' of his account with the processus internus of Versluys. I find no record of the presence of the processus internus in either turtles or snakes; in *Testudo* its absence is definitely affirmed by Bender ('12). It is also lacking in the crocodiles, according to Versluys ('03, revising the statement of his paper of '98) and Shiino ('14). In the vicinity of the processus internus in *Lacerta* Gaupp describes a well-marked ridge extending over the dorsal surface of the stalk of the columella. This ridge is connected with the processus paroticus by the band of connective tissue mentioned in a preceding paragraph. That this ridge is a remnant of the processus dorsalis can hardly be questioned; in *Eumeces* it is absent in all observed stages. Between the processus internus and the insertion plate, the stalk is sharply constricted and vertically flattened, as noted by Versluys ('98) in adult lizards, where the flattening is accompanied by a marked flexibility.

The main part of the insertion plate (fig. 7) may be compared with a stout club, with the larger end pointed obliquely upward

and backward. The point of union with the stalk divides the insertion plate into two unequal parts. The posterodorsal division, *pars superior* (*p.s.*), is short and thick; the anteroventral division, *pars inferior* (*p.i.*), is much more slender, but nearly twice as long. Extending downward and backward from the *pars superior*, close to the union with the stalk, is a well-marked outgrowth, the *processus accessorius posterior* or *processus interhyalis* (*pr.ac.p.*); the opposed *processus accessorius anterior* is barely suggested, if at all, by a slight rounded swelling on the other side of the insertion plate. In stage 6 the *processus accessorius anterior* is clearly lacking. The relatively small size of the *processus accessorius posterior* and the loss of the *processus accessorius anterior* destroy entirely the cruciform appearance of the insertion plate, so striking in Gaupp's figures of *Lacerta*.

A great variety of blastemic, cartilaginous, or ligamentous connections of the *columella auris* with neighboring parts have been described. One of these, that with the quadrate and *crista parotica* through the *processus dorsalis* or its rudiment, has been discussed (p. 156). In *Lacerta* Gaupp describes the *processus internus* as in contact with the quadrate and bound to it by a cord of connective tissue; this connection is affirmed generally for the reptiles by Fuchs ('09), who describes still another connection with the quadrate, leading from the *processus accessorius anterior*. The connections mentioned by Möller ('05) and Peyer ('12) in *Vipera*, and by Bender ('12) in the turtles are seemingly of the latter type. A connection from the *extracolumella* to the lower jaw has been described by Fuchs ('07 a) and Kunkel ('12 b) in *Emys*, and by Shiino ('14) in the crocodile; also earlier by Dollo ('83) and Cope ('85) in lizards, and by Gadow ('88) in *Sphenodon*. The record of Gadow for *Sphenodon* is contradicted by Versluys ('98), and the structure in lizards interpreted as an artifact. Usually, at least, this connection is described as extending from the insertion plate of the *columella* to the retroarticular process of Meckel's cartilage; but in some cases the data are not explicit—in fact, it is far from certain that all of these descriptions refer to the same structure. Another band is described by Versluys ('98) in some adult lizards as extending

from the extracolumella to the pterygoid muscle. Of the various connections of the columella mentioned in this paragraph I can find no suggestion in any stage of *Eumeces*.

Two other connections described by various authors are, however, conspicuously present—from the insertion plate to the processus paroticus and from the processus accessorius posterior to the hyoid arch.

The first of these is probably present in all stages of *Eumeces*, although not clearly recognizable in stage 1 because of the unfavorable direction of the sections. In the younger embryos processus paroticus and insertion plate are relatively closely approximated, and the connection is short; it is also rather thick and of indefinite contour. In later stages the band lengthens strikingly and becomes strongly fibrous in its structure. From the distal end of the processus paroticus it extends downward, forward, and outward, under the posterodorsal end of the quadrate (figs. 11, 13, and 14, *tend.ext.*), and unites with the pars superior of the insertion plate (figs. 12 and 15); beyond this point its course is very easily traced over the lateral surface of the insertion plate, until it gradually disappears near the extremity of the long pars inferior. For a part of the course on the surface of the insertion plate the band is markedly thickened (fig. 16), so as to appear, at first glance, like a nodule of cartilage; nowhere, however, does it show the histological structure of cartilage. This 'tendon of the extracolumella' is carefully described by Versluys ('98) for *Sphenodon* and adult lizards; it was lacking only in *Amphisbaena* among the many lizards studied. Gaupp does not describe or figure it in embryonic *Lacerta*. Versluys originally homologized this structure with the stapedial muscle, but later ('03) rejected this homology.

The connection from the processus accessorius posterior of the insertion plate to the dorsoposterior end of the hyoid arch is of a very different character—a direct continuity, in the cartilage, procartilage, or blastema stage, of the tissue of the columella and hyoid. This is recognizable in my material only in stage 1; here it cannot be questioned. A very careful study of the sections shows a perfectly continuous early cartilage or

procartilage structure passing from one to the other without interruption or histological modification. In stage 2 the cartilages are still so close together that the surrounding condensed connective tissue is confluent. In the later stages the separation becomes gradually greater, and no connective-tissue band remains to suggest the earlier union. This connection was noted in *Lacerta* by Hoffmann ('89), and is confirmed by Gaupp ('00, '05 b), Fuchs ('07 a), and Cords ('09). In *Sphenodon*, according to Versluys ('98, '03), Schauinsland ('00), Howes and Swinnerton ('01), and Fuchs ('09), the union is permanent, although some peculiarities justify a question whether, in this case, it is primitive or secondary. As an embryonic structure it has also been described in *Tropidonotus* by Rathke ('39, cited by Versluys, '98) and Parker ('78), in *Crocodylus* by Parker ('83), and in *Testudo* by Bender ('11). The connection is traced by Kingsley ('00) to the stapes rather than the extracolumella—due apparently to a different identification of the junction of the two parts of the columella in the undifferentiated cartilaginous tissue of the embryo. It is significant that the connection of the columella and hyoid arch is recorded for each of the five main divisions of living reptiles; the frequent negative reports are not to be wondered at, as the connection is usually very transitory. Discussion of its significance is deferred to a later paragraph (p. 162).

The relations of the ramus hyomandibularis of the facial nerve (fig. 16, *n.VII.hy.*) and its branch, the chorda tympani (figs. 16, 17, and 18, *n.VII.c.t.*), to the skeletal parts may be summarized very briefly, as they are identical with those in *Lacerta*. The ramus hyomandibularis passes backward over the stalk of the columella auris (fig. 16), and gives off the chorda tympani. The latter extends forward, almost parallel to the hyomandibular ramus, passing above the 'tendon of the extracolumella,' already described, and the stalk of the columella (fig. 16). It then follows the ventral edge of the quadrate (figs. 17 and 18) for some distance, finally leaving it and bending sharply downward to reach the processus retroarticularis of Meckel's cartilage.

It is with hesitation that I touch upon the question of the origin and nature of the columella auris. Among recent writers,

the great majority make the entire reptilian columella (with the possible exception of part of the insertion plate) a derivative of the hyoid arch—thus Bender ('11, '12), Cords ('09), Gaupp ('98 and later papers, contrary to his earlier view of a double origin), Gregory ('13), Howes and Swinnerton ('01), Kingsley ('00), Kunkel ('11, '12 b), Peyer ('12), Schauinsland ('00), Shiino ('14), Versluys ('98). The view is an old one, having been held by Rathke ('39, cited by Möller, '05). The opposite extreme view, that the columella is of exclusive otic origin, is held by Möller ('05) and Noack ('07). The intermediate view of Parker ('79) and Hoffmann ('89) finds a vigorous supporter to-day in Fuchs ('06 and later papers). According to this view, the columella consists of two genetically distinct divisions—an otostapes, derived from the otic capsule, and a hyostapes, of hyoid origin. As first suggested by Hoffmann and as used by Fuchs, these terms are synonymous with stapes and extracolumella; Versluys ('03), however, who accepts the terms as representing somewhat distinct centers of chondrification, but rejects their genetic significance, uses them in a slightly different sense. According to his view, the division between the permanently cartilaginous extracolumella and the bony stapes of the adult is not coincident with the junction of the cartilage nuclei characterizing hyostapes and otostapes, but is located slightly nearer to the footplate. According to Hoffmann and Fuchs, the processus internus and processus dorsalis belong to the hyostapes; according to Versluys, they belong to the otostapes; all agree as to their extracolumellar nature. Conditions in Eumeces seem to accord with the usage of Versluys, which I shall follow.

Three questions, then, demand consideration: 1. Is the hyostapes genetically related to the hyoid arch? 2. Is the otostapes genetically related to the otic capsule? 3. Are the hyostapes and otostapes genetically related to one another?

Is the hyostapes genetically related to the hyoid arch? As already noted, a structural connection of the hyostapes with the hyoid arch has been observed, at some stage of development, in representatives of all the main groups of living reptiles—Rhynchocephalia, Chelonia, Squamata (both lizards and snakes), and

Crocodylia. The only question is whether this union is primitive or secondary. Only in *Sphenodon* is the union persistent; in most forms it is of very early appearance and disappearance—blastema stage in *Testudo* (Bender, '11); blastema (Cords, '09) or procartilage (Fuchs, '07 a) in *Lacerta*; procartilage in *Eumeces*. In general, too, as in *Eumeces*, the course of development is from complete connection to complete separation. The general occurrence of this connection through the reptilian series, its early ontogenetic appearance, and its later disappearance speak strongly for its primitive character and make very probable the hyoid relationship of the hyostapes.

Is the otostapes genetically related to the otic capsule? That the footplate of the columella and the wall of the otic capsule are in very close union (although usually distinguishable) in early stages of many reptiles is conceded by the supporters of the view that the entire columella is of hyoid origin—Schauinsland ('00) and Howes and Swinnerton ('01) for *Sphenodon*; Versluys ('03) for *Lacerta* and *Hemidactylus*; Cords ('09) for *Lacerta*; Shiino ('14) for *Crocodylus*. On the other hand, in *Platydictylus* and *Gecko* (Versluys, '03) and *Testudo* (Bender, '11, '12) the anlage of the footplate is described as conspicuously distinct from the otic capsule. In the blastema (*Platydictylus* and *Gecko*) or even the cartilage stage (*Testudo*) the otic capsule is said to be completely closed median to, and independently of, the footplate; the fenestra vestibuli arises by a resorption of the tissue of this complete capsular wall. In the geckos Fuchs ('09) has confirmed this distinctness of the anlage of the columella, which he considers a precocious development, due, perhaps, to its perforation by the stapedia artery. *Eumeces* conforms clearly to the first of these types of development. In stage 1 the footplate of the columella is undoubtedly confluent with the surrounding tissue of the capsular wall, although there is a slight histological difference. The columella may perhaps be characterized as procartilage and the surrounding tissue as blastema, but the difference is slight and the transition a very gradual one. Moreover, the superficial parts of the stalk of the columella show

a similar blastemic character, so that a slightly tangential section passes, with no perceptible histologic change, from the stalk of the columella, through the footplate, into the capsular wall. It should also be carefully noted that the above contrast in the histological character of the footplate and the capsule does not apply to the whole of the capsule, but only to that portion immediately surrounding the footplate and destined to develop into the closing membrane of the fenestra vestibuli, the membrana ovalis. This is framed by procartilage identical in its development with that of the columella. In later embryos the contrast of footplate and surrounding membrane becomes ever more conspicuous, the increasing distinctness being due no more to the progressive differentiation of the cartilage of the columella than to the progressive differentiation of the fibrous tissue of the membrane. The development of the cartilage of the rim of the fenestra vestibuli and of the footplate of the columella proceeds *pari passu*; in every stage the two are histologically indistinguishable. This is of importance, in view of the emphasis laid by Bender ('11, '12) and Kunkel ('12 b) upon the differing degree of chondrification of columella and capsule as evidence that the two have no genetic connection. In *Eumeces* there is no such difference. Moreover, this criterion impresses me as essentially unreliable, and leading, if generally applied, to impossible conclusions. Thus the lateral and medial walls of the otic capsule, being of very diverse rates of chondrification, must be independent of one another; again, not only the early formed footplate of the columella, but equally the late-formed filling cartilage of the upper end of the foramen endolymphaticum in *Eumeces*, and, presumably, that of the 'holes,' noted in various parts of the otic capsule by Kunkel himself as well as others and explained as due to retarded chondrification (p. 149), must be interpreted as independent elements. Certainly, the rate of chondrification as a test of genetic relationship must be used with the utmost caution, if at all.

Versluys ('98) and Fuchs ('09) have suggested that the relation of the columella to the periosteum or perichondrium of the otic capsule may be used as a means for determining the origin

of the columella. If the columella is of hyoid origin and has reached the capsule from the outside, clearly the perichondrium of the outside of the otic capsule must be pushed in before the footplate of the columella; on the other hand, if the columella be of otic origin and its footplate has developed in situ, the outer perichondrium of the capsule must also pass continuously over the outer surface of the footplate. This would seem a safe criterion, but it has led Versluys and Fuchs to diametrically opposite conclusions. In the periosteum of adult lizards Versluys finds the first condition general; in the perichondrium of embryos of *Lacerta* and the geckos and in the periosteum of adult *Lacerta vivipara* and *Phyllodactylus*, Fuchs describes the second condition, although recognizing the correctness of Versluys's observations for other adult forms. In this confusion, it seems clear that embryonic conditions are more likely to give a correct clue than are adult conditions—that the adult periosteum is more liable to secondary modification than the embryonic perichondrium. In embryos of *Eumeces* I have found the examination of this point surprisingly difficult. In earlier stages the perichondrium is very indefinite or unrecognizable; cartilage and perichondrium are developing out of a common undifferentiated embryonic matrix. In spite of difficulties, however, the evidence seems to me conclusive that there is no pushing in of an outer perichondrium before the columella, but that the outer covering passes without interruption, as described by Fuchs, over the wall of the otic capsule, the membrana ovalis, and the footplate of the columella.

In *Eumeces* the evidence points consistently to the genetic relationship of otic capsule and otostapes. The confusion of data for other reptiles seems, on the whole, to favor the same view; such cases of striking independence as *Platydictylus* and *Gecko*, rather than the far more numerous cases of close embryonic connection, would appear to be the exceptions demanding special explanation. This interpretation gains additional force from the presumption of at least a partial homology of the reptilian columella and the physiologically corresponding structures of the *Amphibia*, together with the general recognition of

the otic character of the amphibian operculum. All things considered, the otic relationship of the otostapes appears more probable than its independence of the auditory capsule.

Are otostapes and hyostapes genetically related to one another? I discuss this question independently of the answers given to the two preceding questions. In stages 3 to 6 of *Eumeces* the columella is an unquestionably continuous mass of cartilage; but in each of these stages there is equally unquestionably one zone in which the cartilage shows a slightly more embryonic structure—smaller nuclei and less of matrix. This zone, which I interpret as the junction of otostapes and hyostapes, corresponds with the constriction already mentioned between the processus internus and the insertion plate. This is the position assigned to this junction by Versluys, but not by Fuchs (see also p. 162). In stage 2 this zone of junction is procartilaginous rather than cartilaginous, although the balance of the columella has reached the condition of unquestionable cartilage. This is the stage of maximum distinctness of otostapes and hyostapes. In the still younger stage 1 the entire columella is composed of procartilage and no differentiation of the parts is recognizable. This appears to be the usual rule for the development of the columella—an originally continuous anlage, which becomes divided for a very brief period at the beginning of chondrification. Only in *Lacerta*, as described by Fuchs ('07 a), do I find a different succession of events recorded—a division in the blastema stage preceding the continuity of the procartilage. This is not recorded by other workers on *Lacerta*, and for *Emys*, Fuchs himself gives a description apparently consistent with the above account of *Eumeces*. The preponderance of evidence strongly favors the interpretation of the columella as a unit structure, with otostapes and hyostapes in direct genetic relation to one another.

Are the above conclusions consistent? The conclusions that the hyostapes is genetically related to the hyoid arch, that the otostapes is genetically related to the otic capsule, and that the otostapes and hyostapes are genetically related to one another are apparently hopelessly contradictory; I believe that a further

consideration will show that the contradiction is only apparent. The solution of the difficulty is suggested by a phrase of Kingsley ('00), in which he characterizes the columella as developed "from the proximal end of the hyoid arch, or at least from the same continuous stroma." In a similar way I believe that the columella may be said to be developed from the otic capsule "or at least from the same continuous stroma." It is not that the columella, or either of its halves, is a formal outgrowth from a previously developed otic capsule or hyoid arch; rather that otic capsule, columella auris, and hyoid arch are all parts of a 'continuous stroma' of undifferentiated early embryonic tissue. In this, as suggested by Gaupp for another portion of the head skeleton (see also p. 130), the potentiality of cartilage development in situ is generally distributed. Thus the otostapes may develop in connection with the otic capsule and the hyostapes in connection with the hyoid arch, and yet hyostapes and otostapes may form a genetic unit in the columella auris. This unity is no more disturbed by the presence of somewhat distinct otostapedial and hyostapedial centers of chondrification than is the unity of the otic capsule itself by the fact that its chondrification does not proceed uniformly in all parts, but from rather distinct centers. On this interpretation the discussion of the otic or hyoid nature of the columella auris becomes like the classic dispute concerning the gold and silver shield. On one side the columella is of otic relationship, on the other it is of hyoid relationship, but it does not thereby give up its unit character. This is not offered as a dogmatic statement of unquestioned fact, but rather presented as the tentative result of the study of the development of the columella in Eumeces and an examination of the very confused and confusing literature dealing with the reptilian columella.

6. ORBITOTEMPORAL REGION

1. *General description*

In his *Lacerta* paper ('00) Gaupp applied the name 'regio orbitalis' to the entire section of the skull between the otic and ethmoid regions; later ('05 b) he introduced the more adequate term 'regio orbitotemporalis.' The orbital and temporal subregions (figs. 1, 2, and 3), which together make up the orbitotemporal region, differ from one another as regards the extent of the enclosed brain cavity, the character of the cranial floor, and the character of the lateral walls; they resemble each other in the entire lack of a cartilaginous cranial roof.

In dorsal view the brain cavity in the orbitotemporal region is strongly pear-shaped, with the stem end turned forward. In the narrow orbital portion are located the olfactory lobes of the brain; the enormously expanded temporal portion contains the cerebral hemispheres and optic lobes. It is hardly necessary to note that these statements can be only general; the morphological divisions of the brain do not correspond exactly with the regional divisions of the skull. The contrast in the width of the orbital and temporal regions of the cranium is much more conspicuous in *Eumeces* than in *Lacerta*. In Gaupp's figure 1 the ratio of the maximum width of the temporal region to that of the orbital is barely more than two to one; in *Eumeces* the corresponding measurements give a ratio of at least three to one. In the temporal region the cranial floor is composed of the two trabeculae (fig. 2, *trab.*), the paired structure of which may be recognized even in their fused anterior parts; in the orbital region, on the other hand, the floor consists of the unpaired interorbital septum (figs. 2 and 3, *sep.i-o.*). The side walls of the temporal region are very rudimentary, consisting of a latticework of slender rods and bars of cartilage, while the walls of the orbital region are formed of the continuous plates of the solum supra-septale (figs. 1 and 3, *sol.s-s.*). The rudimentary character of the lateral wall of the temporal subregion is much more marked in *Eumeces*, at least in the later stages, than in *Lacerta*.

The connections of the orbitotemporal region with the posterior and anterior portions of the skull are rather scanty. Posteriorly it is united with the basal plate by means of the trabeculae, and with the otic capsules by means of the taeniae marginales (fig. 3, *t.marg.*); anteriorly the interorbital septum is continuous with the nasal septum, and the cartilagine sphenothmoidales (fig. 1, *c.sph-e.*) connect the solum suprasedale with the roof of the nasal capsule.

For detailed discussion it will be convenient to consider first the floor of the entire orbitotemporal region, postponing till later the separate consideration of the lateral walls of the two subregions. This division of floor and lateral walls is convenient but arbitrary; the solum suprasedale, for example, aids conspicuously in the formation of the floor of the brain cavity, although belonging primarily to the lateral wall.

2. Floor of entire orbitotemporal region

The two trabeculae (fig. 2, *trab.*) arise from the front edge of the basal plate, immediately under the abducens foramina. As slender rounded rods they extend forward and inward and meet in the middle line of the skull. As seen from the side (largely concealed in figure 3 by the processus basipterygoideus, *pr.b-pt.*), the course of the trabeculae is first obliquely downward, then obliquely upward, and finally, in the region of their fusion, almost horizontal. The upward bend of the middle course is indicated by the elongate form of the section of the trabeculae (*trab.*) as seen in figure 22. In conjunction with the basal plate, the trabeculae surround an approximately triangular opening in the cranial floor, the fenestra hypophyseos (figs. 1 and 2, *fen.hyp.*), largely occupied by the hypophysis. In the middle line the curved anterior margin of the basal plate encroaches somewhat upon the fenestra, thus emphasizing its posterolateral angles, the incisurae caroticae of Gaupp, through which the internal carotid arteries enter the skull. Conditions here are identical with those in *Lacerta*. This course of the carotids is characteristic of the reptiles in general—snakes (Peyer, '12), crocodiles (Shiino, '14), turtles (Fuchs, '12; Kunkel, '12 b); it is also

essentially duplicated in embryonic *Echidna* (Gaupp, '08 a). In the higher mammals the carotids enter the skull lateral to the homologue of the trabeculae and independently of the fenestra hypophyseos; conditions in the marsupials are described by Broom ('09) as intermediate between those in *Echidna* and in the placentals. In the reptiles there are minor variations. In *Emys*, Kunkel ('12 b) reports the absence of definite incisurae caroticae; on the other hand, Fuchs ('12) describes the original fenestra hypophyseos of *Chelone* as divided by a transverse bar of cartilage and the longitudinal intertrabecula into an anterior definitive fenestra hypophyseos, which is soon obliterated, and two posterior foramina for the carotid arteries. The independent carotid foramina of *Chelone* were noted by Gaupp ('05 b). Nick ('12) also describes an intertrabecula in *Chelone* and *Dermochelys*; Parker ('80, '83) has recorded the same structure in *Chelone* and the crocodile. It is entirely lacking in *Eumeces*.

For a short distance in front of the fenestra hypophyseos the trabeculae lie in close contact, but are not actually fused with one another (fig. 23, *trab.*); they then unite gradually but completely, and are continued forward in the thickened basal margin of the high interorbital septum (fig. 3, *sep.i-o.*) characteristic of the orbital region of reptiles generally. Only in the snakes is the septum reduced to a connective-tissue plate (Gaupp, '05 b) or entirely lacking (Parker, '78; Peyer, '12). In *Emys*, Kunkel ('12 b) describes a conspicuously paired structure of the septum; in *Eumeces* any suggestion of such a paired character is confined to the ventral margin and the extreme posterior end—the region of transition from the trabeculae. According to Schauinsland ('00) and Howes and Swinnerton ('01), the trabeculae are much less closely united with the interorbital septum in *Sphenodon*, especially in younger embryos, than in *Eumeces*, and in *Chelone* Fuchs ('12) denies any participation of the trabeculae in the formation of the septum.

The posterior free margin of the interorbital septum (fig. 3) is broken by two notches—a lower narrow incision just above the trabeculae and a much larger indentation occupying the upper two-thirds of the margin of the septum; between the two is a

small remnant of the septum, the cartilago hypochiasmatica (fig. 3, *c.hyp.*), over which the optic nerves cross in passing to the orbits (fig. 23, *n.II.ch.* and *c.hyp.*). In *Dermochelys* Nick describes a closed fenestra in place of the more characteristic notch between the cartilago hypochiasmatica and the fused trabeculae; a similar condition is noted by Kunkel ('12 b) as a temporary stage in the formation of the notch in *Emys*. The cartilago hypochiasmatica is described by Gaupp as cylindrical ('drehrund') in *Lacerta*; in *Eumeces* it is a flattened vertical plate. A minute transverse perforation appears to have no special significance. The connection of the cartilago hypochiasmatica with the subiculum infundibuli and the lateral wall of the temporal subregion is discussed later (p. 176).

In stage 5 of *Eumeces*, as in *Lacerta*, the septum interorbitale is not a continuous structure, but is interrupted by an enormous fenestra (figs. 3 and 24, *fen.sep.*) in its upper portion just below the union with the solum suprasedale. In fact, so far as the septum itself is concerned, this is a deep dorsal notch rather than a true fenestra, for its upper boundary, in the posterior part, is formed not by the septum, but by the solum suprasedale (fig. 24). In *Lacerta* Gaupp figures another fenestra a little further forward, just at the transition from septum interorbitale into septum nasale. If there be such a second fenestra septi in stage 5 of *Eumeces*, it is very minute and limited to one or two sections; at best, its presence is questionable. On the other hand, there is unquestionably an area in which the cartilage of the septum is conspicuously thinned; in a later embryo, stage 6, this thinner area is replaced by a fenestra of considerable size. The position of this thin area or fenestra is rather more anterior in *Eumeces* than in *Lacerta*, falling in the ethmoid rather than the orbital region. It is improbable that these fenestrae have any essential morphological significance, but as the external signs of the forces influencing the development of the skull they may be of considerable import. In this connection further comparison of different developmental stages proves interesting. In stage 5 a second thin spot is noted in the septum, immediately below the principal fenestra septi (fig. 24), and in

stage 6 the cartilage is almost perforated at this point. In stage 4 the principal fenestra is present, but the other fenestrae (or thin spots) of stages 5 and 6 are unrecognizable. Finally, in stage 2, there is no fenestration; the septum, interorbital and nasal, extends as an uninterrupted plate from the cartilago hypochiasmatica to the tip of the nose. Thus the septum is already in regressive development in the later stages of Eumeces. It is a very natural suggestion that this degeneration and fenestration of the septum interorbitale are but a later step in its development—that the very forces which have displaced the brain upward and led to the formation of the septum have by their continued activity also caused the resorption of parts of the earlier continuous cartilage. As chief among these forces we may well follow Gaupp in emphasizing the pressure exerted by the enormously developing eyes. In *Sphenodon Schauinsland* ('00) and Howes and Swinnerton ('01) describe a similar transition from an early imperforate to a later fenestrated condition of the septum. Other data are isolated and have no bearing on the present point. In *Chelydra* Nick describes three fenestrae, in *Chelone* and *Dermochelys* none. Kunkel ('12 b) also describes an imperforate septum in *Emys*, and Shiino ('14) in *Crocodylus*.

The solum suprasedale belongs alike to the floor and the lateral wall of the cranium in the orbital region. Its consideration is more convenient in the latter connection (p. 181).

3. Basispterygoid process and associated structures

At the posterior limit of the temporal region is located a group of structures closely associated with one another and with the pterygoid bone, which may well be described together at this point, although of diverse origin and relations—the processus basispterygoideus, cartilago articularis ossis pterygoidei or meniscus pterygoideus, processus pterygoideus quadrati, and epipterygoid. In adopting Parker's ('79 and earlier) term 'epipterygoid' for the latter structure, in preference to 'antipterygoid' (suggested by Gaupp, '91 a, and still used in '00), I follow

Gaupp's later usage ('05 b). The epipterygoid has also frequently been known by the very unsatisfactory Cuvierian name, 'columella' or 'columella cranii,' all too easily confused with the 'columella auris.'

The basipterygoid process (fig. 2, *pr.b-pt.*) arises from the anterior margin of the basal plate, just lateral to the trabecula (fig. 21, *pr.b-pt.* and *trab.*). Its form is that of a very oblique capital T, with cylindrical stem and horizontally flattened cross bar. The stems of the two processes extend laterally as well as anteriorly, diverging strongly from one another; the cross bars lie almost parallel in an anteroposterior direction. The distal portion of the processus basipterygoideus comes into close relation with the pterygoid bone (fig. 2), articulating with it by means of an interpolated disc of cartilage, the cartilago articularis or meniscus pterygoideus (figs. 1, 3, and 22, *c.art.*). The epipterygoid (fig. 3, *epipt.*) is a slender rod of cartilage with its enlarged lower end or foot (fig. 21, *epipt.*) resting in a well-marked dorsolateral groove in the pterygoid bone (*os pt.*) just opposite the posterior end of the cross bar of the basipterygoid process (*pr.b-pt.*) and slightly posterior to the articular cartilage. From this point the rod extends obliquely upward, backward, and outward to terminate just above the anterior ampullar prominence of the otic capsule. Just in front of the foot of the epipterygoid, and also in a dorsolateral groove of the pterygoid bone, lies another much smaller cartilaginous rod, the processus pterygoideus quadrati (figs. 1, 3, 22, and 23, *pr.pt.*).

The much longer processus pterygoideus of *Lacerta* is described as showing a sudden lateral bend near its anterior end. In *Eumeces* the rod is nearly straight; but a little in front of it, and decidedly lateral, there is a curious little nodule of cartilage (figs. 1, 2, and 3, *pr.pt'*), by no means constant in its occurrence. In stage 5 it is present on each side; in stage 6 it is more strongly developed on one side and lacking on the other; in stage 4 and earlier stages I am unable to distinguish it. From its position, especially in stage 6, and its association with the lateral anterior arm of the Y-shaped pterygoid bone (fig. 24, *pr.pt'* and *os.pt.*), it can hardly be questioned that this nodule represents the rudi-

mentary anterior end of the pterygoid process described in Lacerta. Schauinsland ('03) figures a similar fragmentation of the anterior end of the processus pterygoideus in a late stage of *Sphenodon*, and describes the entire process as very long, extending forward from the pterygoid bone upon the transversum (not palatinum, as erroneously stated in an earlier paper, '00).

In Lacerta Gaupp describes the pterygoid process as continuous with the epipterygoid. This is not the case in the later stages of *Eumeces*, in which these two cartilages are clearly distinct, but in stage 2 the continuity is beyond question. In this stage I am also unable to distinguish the articular cartilage as an element distinct from the basipterygoid process. The two are represented by a confluent mass of procartilage, which also connects, dorsally to the pterygoid bone, with the more advanced cartilage of the epipterygoid and pterygoid process. Gaupp ('05 b) has also noted the connection of the rudiments of the basipterygoid process, articular cartilage, and epipterygoid in early stages of Lacerta.

The processus basipterygoideus is well developed in lizards and *Sphenodon* (Schauinsland, '00; Howes and Swinnerton, '01); Gaupp ('12) reports that it is present in some snakes. In turtles it was long unrecognized, but has been described, in greater or less development, in *Emys* (Fuchs, '10; Gaupp, '10; Kunkel, '11, '12 b), *Podocnemis* (Gaupp, '10), *Chelone* (Fuchs, '12), and *Chelydra* (Nick, '12). It is probably present in other species in which it has not yet been observed. In the crocodiles it is apparently wanting, although Gaupp ('05 b) suggests its possible presence in very rudimentary form—"eine Andeutung scheint vorhanden zu sein." Shiino ('14) denies its presence, emphasizing the substitution of an avian processus basitrabecularis for the more reptilian processus basipterygoideus. The structure is thus very widely distributed in the reptilian series; its absence in the Crocodilia Gaupp ('12) interprets as due to degeneration. The homology of the basipterygoid process with the ala temporalis of the mammal, proposed and discussed in detail by Gaupp ('00), is generally accepted, at least as later

restricted by Gaupp himself ('08 a, '12) to the basal portion, processus alaris, of the ala temporalis. There is nothing in my work to throw new light on this point, as the form and relations of the processus basipterygoideus are essentially identical in *Lacerta* and *Eumeces*.

Epipterygoid, articular cartilage, and pterygoid process are considered as belonging to the dorsal portion of the first visceral arch, and will be further discussed in that connection (p. 200).

4. *Lateral wall of temporal subregion*

The lateral wall of the temporal subregion is reduced to a mere latticework, as in *Lacerta*, but in no single stage examined is there any such completeness of this lattice or any such geometrical regularity in the arrangement of the various fenestrae as figured by Gaupp. By the combination of different stages of *Eumeces* it is possible to identify each element of the lateral wall of *Lacerta*, with the single exception of the rather anomalous supratrabecula, which Gaupp ('05 b) describes as occurring on one or both sides of some specimens of *Lacerta*. In early stages of *Eumeces* two additional bars of cartilage are found which are not mentioned by Gaupp.

In stage 5 of *Eumeces* the taenia marginalis (figs. 1 and 3, *t.marg.*) extends without interruption from the uppermost part of the otic capsule to the posterolateral margin of the solum suprasedale. The union at each end is a secondary one, as is seen by a comparison with stage 2 (fig. 33), in which the developing taenia is still unattached both posteriorly and anteriorly. For a possible continuation of the taenia marginalis into the orbital region, see page 182. In the middle third of its course the taenia marginalis makes a great lateral curve (fig. 1), marking the widest point in the cranium, as already noted.

In addition to the taenia marginalis, only the cartilages surrounding the fenestra optica show a development in stage 5 of *Eumeces* corresponding to that described for *Lacerta*. From the lower posterior corner of the solum suprasedale on each side a narrow band of cartilage extends backward, nearly parallel

to the taenia marginalis. After a short course this cartilage band, the taenia parietalis media (fig. 3, *t.par.m.*), connects with one apex of a somewhat extensive triangular plate of cartilage. A second apex of this plate extends downward and toward the middle line as a flat band of cartilage and unites with its fellow of the opposite side. Thus is formed the subiculum infundibuli (figs. 1 and 3, *sub.inf.*), located just above the anterior fusion of the trabeculae, and connected with the septum interorbitale by means of the cartilago hypochiasmatica (fig. 3, *c.hyp.*) already described. The subiculum infundibuli and the anterior portions of the taeniae parietales mediae form on each side a nearly semicircular lateroposterior boundary for a large opening, the fenestra optica (fig. 3, *fen.op.*), whose median boundary, common to the fenestrae of the two sides, is formed by the posterior margin of the septum.

From the third apex of the triangular plate in which subiculum and taenia parietalis media unite, a band of cartilage extends backward, continuing nearly the course of the taenia parietalis media, but bending slightly upward. Near its posterior end (almost in contact with it on one side of the specimen modeled) is an isolated plate of cartilage of irregularly triangular form (fig. 3, *t.par.m'*).

Of pila accessoria and taenia parietalis media, posterior to the subiculum infundibuli, there is no sign in stage 5 of Eumeces, unless the rudimentary cartilages just mentioned can be homologized with some of these structures. The pila prootica is reduced to the merest rudiment. In stage 2, on the other hand, all of these elements are clearly recognizable. Figure 33 represents a graphic reconstruction of the lateral wall of the temporal region in this stage. In the reconstruction the interpretation has been liberal, and some parts represented are undoubtedly procartilage rather than true cartilage. Pila accessoria (*pi.ac.*) and pila prootica (*pi.pr.ot.*) are identically as in *Lacerta*, except for a slight interruption of the pila accessoria on one side, not shown in the figure. The taenia parietalis media (*t.par.m.*) extends backward, as in *Lacerta*, to connect with the pila prootica and enclose the fenestra metoptica (*fen.m-op.*). In *Lacerta*

the taenia parietalis media stops at this point, but in this early embryo of Eumeces it can be clearly traced backward and upward to a union with the taenia marginalis, as figured. Thus the fenestra prootica is divided into two fenestrae—an antero-dorsal and a posteroventral, designated respectively as fenestra prootica superior (*fen.pr-ot.s.*) and fenestra prootica inferior (*fen.pr-ot.i.*). A further addition to the skeletal plan of Lacerta is seen in the bar, marked *x* in the figure, which again subdivides the fenestra prootica superior. This bar is exceedingly thin and of rather questionable structure; it is not deemed worthy of a special name. The scanty development of the taenia marginalis in this stage leaves the fenestra prootica inferior and the fenestra epiotica (*fen.e-op.*) dorsally incomplete.

From the same diagram it is easy to trace the probable homology of the cartilage spur projecting posteriorly from the junction of subiculum infundibuli and taenia parietalis media in stage 5, as well as the isolated fragment near its tip. The representation of stage 5 in this figure is not exact nor drawn to scale; all portions common to the two stages are represented as in stage 2, but the relation to nerve exits makes possible a close approximation to accuracy as regards the two cartilages under consideration. Both belong to the taenia parietalis media, the isolated fragment falling wholly or mainly in the posterior extension (*t.par.m'*) which is lacking in Lacerta.

Of especial interest in this comparison of earlier and later stages of Eumeces is the fact that different parts of the lateral wall show very diverse degrees of development. For the most part, the latticework is more complete in the younger embryos. But, while the major part of the lateral wall in stage 5 has suffered a marked ontogenetic regression, the taenia marginalis is still developing progressively and has only just reached its complete extension. An exactly parallel progressive development of the taenia marginalis after other portions of the temporal wall are already in regression is shown in the figures of Sphenodon by Howes and Swinnerton ('01). These observations may well have considerable phylogenetic significance in comparison with the Mammalia. Here the commissura orbitoparietalis, which is

homologized with the *taenia marginalis*, and for which Fischer ('01 b) retains the latter name, is usually highly developed. On the other hand, the lateral extension of the *cavum cranii* has led to the development of a secondary lateral wall and the further degeneration of the primary wall. This is usually described as wholly lost, but some rudiments of the original structure have been recognized by Voit ('09 a, '09 b) in *Lepus*, by de Burlet ('13 b, '13 c, '14 a, '16) in *Cetacea*, and by Terry ('17) in the cat.

The lateral wall of the temporal region shows the same essential structure throughout the entire reptilian series, although there are striking variations in detail and, in some cases, extreme reduction. In *Sphenodon* (Schauinsland, '00; Howes and Swinerton, '01) the wall is very solid and has the appearance of a fenestrated plate rather than a latticework. Even here the majority of the cartilaginous elements and fenestrae of *Lacerta* and *Eumeces* may be identified. Also in the crocodile (Shiino, '14) the cartilages are heavy and the fenestrae of restricted extent. In the turtles there is extreme variation. Nick ('12) reports a very complete wall in *Dermochelys* and a very rudimentary one in *Chelydra*; *Chelone* is intermediate. Emys, according to Kunkel ('12 b), shows the rudimentary type of structure, the *taenia marginalis* lacking its connections, alike with *solum suprasetale* and with *otic capsule*. The extreme of reduction is reached in the snakes, where the lateral wall is practically wanting (Gaupp, '05 b, '06; Peyer, '12).

5. *Fenestrae of temporal subregion*

In figure 33, graphically reconstructed from stages 2 and 5, the cartilages of the lateral wall of the temporal region are represented, each in its maximum development. If the band of questionable cartilage (*x*), extending from the *taenia marginalis* to the *taenia parietalis media*, just posterior to the *pila accessoria*, be ignored, five great fenestrae may be recognized. Two of these, the *fenestra epiptica* (*fen.e-op.*) and the *fenestra proptica superior* (*fen.pr-ot.s.*), are in an upper row, dorsal to the *taenia*

parietalis media; two, the fenestra optica (*fen.op.*) and the fenestra metoptica (*fen.m-op.*), are in a lower row, ventral to the taenia parietalis media; the fifth, the fenestra prootica inferior (*fen.pr-ot.i.*), extending from the basal plate to the taenia marginalis, may be interpreted as common to both rows. The fenestrae of the two rows are of widely different significance. Those of the upper row, including the dorsal part of the fenestra prootica inferior, are without relation to important organs; on the other hand, each fenestra of the lower row, including the ventral part of the fenestra prootica inferior, affords exit for one or more of the cranial nerves. As the relations are almost identical with those in *Lacerta*, they demand only brief summary. The names of the fenestrae are unchanged from Gaupp, except for the specification, as superior and inferior, of the two fenestrae into which the fenestra prootica is divided by the posterior continuation of the taenia parietalis media. For simplicity, only the four terms, anterior, dorsal, posterior, ventral (always in this order), are used in bounding the fenestrae, the usually more or less conspicuous obliquity and other irregularities of the boundaries being disregarded.

Fenestra epioptica (*fen.e-op.*), bounded by the posterior edge of the solum suprasettale (anterior), the taenia marginalis (dorsal), the pila accessoria (posterior), and the taenia parietalis media (ventral).

Fenestra prootica superior (*fen.pr-ot.s.*), bounded by the pila accessoria (anterior), the taenia marginalis (dorsal), the taenia parietalis media (posterior and ventral). The subdivision of this fenestra by a band of questionable cartilage (*x*) has been noted.

Fenestra optica (*fen.op.*), bounded by the edge of the septum interorbitale (anterior), the taenia parietalis media (dorsal), the subiculum infundibuli (posterior), and the cartilago hypochiasmatica and the edge of the septum interorbitale (ventral). As indicated by the name, this fenestra serves as the exit of the optic nerve (*n.II.*).

Fenestra metoptica (*fen.m-op.*), bounded by the subiculum infundibuli and cartilago hypochiasmatica (anterior), the taenia

parietalis media (dorsal), the pila prootica and corresponding upward projection of the basal plate (posterior), and the trabecula (ventral). Through this opening the oculomotor (*n. III.*) and trochlearis (*n. IV.*) nerves leave the cranial cavity, as described in *Lacerta*; also the abducens (*n. VI.*), discussed in an earlier paragraph (p. 134).

Fenestra prootica inferior (*fen.pr-ot.i.*), bounded by the pila prootica (anterior), the taenia parietalis media and taenia marginalis (dorsal), the otic capsule (posterior), and the basal plate (ventral). The dorsoposterior portion of this very irregular fenestra contains no important organs, but through the lower portion, incisura prootica, the trigeminus nerve emerges by two main trunks (*n.V.1.* and *n.V.2-3.*), each swelling into a large ganglion mass. The two ganglia (fig. 19, *g.V.a.* and *g.V.p.*), rather well separated from one another as in *Lacerta*, but not in *Emys* (Kunkel, '12 b), largely fill the pocket formed between the pila prootica, otic capsule, edge of basal plate, and epipterygoid. The three main branches of the nerve are distributed as in *Lacerta*, the first (fig. 21, *n.V.1.*) arising from the anterior ganglion and passing forward, above the processus basiptyergoideus and between the pila prootica and epipterygoid, while the second and third (figs. 19 and 21, *n.V.2.* and *n.V.3.*) leave the posterior ganglion in a more lateral direction and are separated from the first by the cartilaginous rod of the epipterygoid.

In stage 5 only the fenestra optica (fig. 3, *fen.op.*) is fully retained. Posterior to this the lateral wall has become so rudimentary that the fenestra epioptica, fenestra metoptica, and fenestrae prooticae, superior and inferior, have become confluent as a single enormous fenestra (fig. 3 *fen.y.*), whose subdivision is barely suggested by the cartilage remnants already described.

The variation in the reptilian series as regards the degree of completeness of the lateral temporal wall necessarily leads to corresponding differences in the fenestrae. Most interesting is the occasional division of the fenestra metoptica in such a way that the oculomotor and trochlearis nerves are provided with independent foramina. This has been recorded for *Chelone* by Gaupp

('05 b) and for *Dermochelys* by Nick ('12); in *Emys*, Kunkel ('12 b) reports a distinct notch for the oculomotor in the margin of the fenestra metoptica. Nick holds the division of the fenestra metoptica to be the primitive condition—a view which gains force from the observations of Schauinsland ('00) and Howes and Swinnerton ('01) on *Sphenodon* in which the originally separate foramina later become confluent in a fenestra metoptica essentially like that of the lizards. For the crocodiles the data are contradictory, but the contradiction is probably due in part to differences in the age of the embryos studied. Gaupp ('05 b) finds separate openings for both nerves, but cites Parker ('83) as authority for their exit through a common fenestra metoptica. Shiino ('14) agrees with Parker as regards the oculomotorius, but describes the trochlearis as emerging either through a separate foramen or through the fenestra optica—never through the fenestra metoptica. Shiino also emphasizes the closure of the fenestra prootica in the crocodiles, and seemingly holds the union of the taenia marginalis and otic capsule unique in this group, in contrast to *Sphenodon*, lizards, snakes, and turtles. The closure of this fenestra by the fusion of the taenia marginalis with the otic capsule is clearly described or figured in late stages of *Sphenodon* (Howes and Swinnerton, '01. Schauinsland, '00, describes an earlier stage in which the fenestra is still open), in *Dermochelys* (Nick, '12), and in *Lacerta* (Gaupp, '00). Only in the snakes, where there is practically no lateral wall, is the fenestra prootica never closed dorsally.

6. *Lateral wall of orbital subregion*

In striking contrast to the latticework of the temporal subregion, the lateral wall of the orbital subregion consists on each side of a single plate of cartilage, sloping downward to the upper edge of the septum interorbitale, thus forming a sort of trough, the solum suprasedale (fig. 1, *sol.s.s.*), for the support of the olfactory lobes and the anterior part of the cerebral hemispheres. That the two plates of the solum fuse rather with the septum than with one another (as already noted by Gaupp) is

clearly indicated in my sections. Posterior to the fenestra septi the limits of the three elements are not well marked, as the fusion is very complete; above the posterior part of the fenestra the two plates of the solum (fig. 24, *sol.s-s.*) appear distinct, although closely approximated. They are separated only by connective tissue; the fenestra is thus rather a notch in the upper edge of the septum, as it is closed dorsally by the solum alone. A little further forward a rather indefinite mass of cartilage is interpolated between the plates of the solum, although apparently distinct from each; still further forward, at the limit of the fenestra, this cartilage may be clearly traced into the recompleted septum. Gaupp's interpretation of the solum as a paired anlage is thus well corroborated by my observations.

The posterior third of the solum is broad, the anterior two-thirds narrow (fig. 3.), but the transition is not so sudden nor the difference so striking as in *Lacerta*. The upper edge of the broader posterior portion is noticeably thickened and may well be interpreted as the forward continuation of the taenia marginalis of the temporal region. In *Lacerta* this thickened margin is carried somewhat further forward as a freely projecting spur alongside of the narrowing solum; this forward projection is not recognizable in stage 5 of *Eumeces*, but is very apparent in other series, both earlier and later. The thin plates of the solum are more or less fenestrated; some of the larger fenestrae are shown in figures 1 and 3, but the smaller ones were lost in the modeling. A similar fenestration of the solum suprasedale is recorded by Kunkel ('12 b) in *Emys* and by Nick ('12) in *Dermochelys*, *Chelone*, and *Chelydra*. Nick suggests a possible homology of these perforations with the fenestra epiptica of *Lacerta*; their occurrence together with the fenestra epiptica in *Eumeces* speaks against this view, while their extreme irregularity indicates that they are without special significance.

Anteriorly the narrow plates of the solum suprasedale, freeing themselves from the interorbital septum, pass continuously into the cartilagine sphenothmoidales (fig. 1, *c.sph-e.*) of the ethmoid region, discussed on page 184.

7. ETHMOID REGION

1. *General description*

The ethmoid region is very complicated and difficult to describe. It is also a region peculiarly subject to secondary modification in connection not only with the olfactory function, but also with the mechanical uses of the snout; moreover, as pointed out by Gaupp ('06) and Kunkel ('12 b), the structure of the ethmoid region is closely correlated with the function and structure of the jaws. It is presumably the type of jaw development which has determined the completeness and solidity of the nasal capsule in crocodiles (Gaupp, '05 b; Shiino, '14) and turtles (Gaupp, '05 b; Ogushi, '11; Nick, '12; Kunkel, '12 b) and its incompleteness and delicacy in snakes (Gaupp, '05 b; Peyer, '12). The lizards are intermediate between these extremes.

In the ethmoid region the formal differences between stage 5 of Eumeces and Gaupp's figures of *Lacerta* are very conspicuous. Some of these differences are reduced or disappear in a later stage of Eumeces; others, while conspicuous, are of little real significance; one, the more complete development of the lateral wall in the extraconchal portion of the capsule, seems constant in all stages and not without importance.

In general, the ethmoid cartilages of Eumeces are of late development in comparison, for example, with those of the lateral temporal wall. The latter, with the exception of the taenia marginalis, are already in regression in stage 5; the former, with the exception of the nasal septum, are not yet fully developed.

The dorsal view (fig. 1) is best adapted to general orientation. The main outline, disregarding fenestrae, may be compared to that of a heart, with the widely expanded atria turned toward the posterior, and the ventricles, slightly divergent at their tips, forming the end of the snout. The groove between the atrium and ventricle of each side is represented by the deep infolding of the aditus conchae (*ad.co.*), the auricle itself by the extraconchal portion of the capsule (*p.ext.*). Posteriorly, between the two extraconchal portions, the roof of the nasal capsule ter-

minates with a curved margin, which forms the anterior limit of the enormous fenestra olfactoria (*fen.ol.*). The lateral boundaries of this fenestra are formed by the sphenethmoid cartilages (*c.sph-e.*), converging backward from the lateral margins of the nasal roof to unite with the narrow anterior end of the solum suprasedale of the orbital region. Extending through the middle of the fenestra olfactoria is the free dorsal margin of the septum, in the region of transition from interorbital to nasal, dividing the fenestra into symmetrical right and left halves, and in each of these halves is the vertical wall which forms the true posterior limit of the nasal region—the planum antorbitale (*pl.ant.*). Septum and sphenethmoid cartilages form the sole connection of the ethmoid region with the posterior regions of the skull.

It is convenient to divide the ethmoid region transversely into three parts of approximately equal length. This threefold division is an essentially morphological one and corresponds with that employed by Gaupp for *Lacerta* and the *Amphibia*. The posterior third, determined by the extraconchal recesses, is much the broadest of the three. Its real division from the middle third is marked by the depth of the conchal infolding, into which the aditus conchae leads, although laterally the extraconchal portion extends considerably further forward, overlapping with the middle third of the ethmoid region. The anterior third is related to the external nostrils, and is the narrowest of the three regions. It is divided from the middle third by a rather conspicuous lateral notch, just behind the superior alar process (fig. 3, *pr.al.s.*). Between this notch and the conchal infolding the middle third expands to a width intermediate between that of the anterior and that of the posterior third. This region is the *zona annularis* of Gaupp. The enormous fenestrae superiores nasi (fig. 1, *fen.s.na.*) lie mainly in this division, but extend slightly into the anterior third.

2. Nasal septum.

At the transition from the orbital to the ethmoid region the nasal septum is a comparatively low vertical plate with free dorsal and ventral margins (fig. 3, *sep.na.*), but it increases rather suddenly in height to meet the nasal roof or tectum. From this point to the tip of the snout its height falls off gradually, its dorsal margin showing a rather uniform curve, corresponding to the profile of the snout. Between the very large and confluent fenestrae superiores nasi the dorsal margin of the septum is free (figs. 1, 27, and 28). This is not the case in *Lacerta*, where the fenestrae are much smaller, but it holds true for all observed stages of *Eumeces*. Elsewhere the septum is fused with the tectum, except, perhaps, for a very short distance at the very tip of the nose, where the foramina apicalia (fig. 1, *f.ap.*) are very closely approximated and separated certainly by little more than the septum itself. The ventral margin, on the other hand, is free for the greater part of its length, fusing only at the extreme anterior end with the very degenerate solum nasi (fig. 2, *sol.na.*). Through a considerable distance the lower edge of the septum is closely bordered by the paraseptal cartilages (figs. 2, 25, and 26, *c.par.*), but in no case is there actual contact. The lower outline of the septum is ventrally concave, continuing almost exactly the curvature of the corresponding margin of the interorbital septum. The curvature of the ventral edge is decidedly less than that of the dorsal.

The ventral edge of the posterior portion of the septum nasale, like that of the septum interorbitale, is decidedly thicker than its upper part (figs. 25 and 26). Further forward this thickening disappears. In this region in *Lacerta* Gaupp describes a thickening of the septum in middle height, forming a low longitudinal ridge on each side, which supports the median edge of the septomaxillary bone. This ridge is very slightly developed in *Eumeces*, and is recognizable only in the later stages. In stage 6 the septomaxillary (fig. 27, *os s-max.*) is very closely approximated to the septum, and, for some distance, comes into actual contact with this slight thickening, but in earlier stages, even stage 5

(fig. 28), the bone is everywhere separated from the septum by a considerable space, decreasing with the age of the embryo. In *Emys* this longitudinal ridge is well marked, according to Kunkel ('12 b), and closely paralleled by a longitudinal rod of cartilage, the 'pila supraglandularis.' The latter structure is not found in *Eumeces* nor mentioned by Gaupp in *Lacerta*.

In stage 5 of *Eumeces* the nasal septum is uninterrupted, although a thinner area in the region of the planum antorbitale suggests the anterior fenestra septi of *Lacerta*. In earlier stages not even this suggestion is present, while stage 6 shows an opening of considerable size. The significance of these observations has been discussed in connection with the interorbital part of the septum (p. 171). A similar, but very variable, perforation of the septum nasale is described in a number of turtles—*Trionyx* (Ogushi, '11), *Emys* (Kunkel, '12 b), *Chelonia* and *Dermochelys* (Nick, '12). In *Chelydra* Nick was unable to distinguish a fenestra. This fenestration is probably wholly independent of the development of a fenestra (or thin spot) in the extreme anterior portion of the septum of various mammals, e.g., *Echidna* (Wilson, '00; Gaupp, '08 a), *Ornithorhynchus* (Wilson, '00), *Talpa* (Fischer, '01 b), *Sus* (Mead, '09), interpreted, after Spurgat ('96, cited by Mead), as correlated with the flexibility of the snout.

Of the prenasal extension of the septum, described in *Chelone* (Parker, '80; Nick, '12), *Chelydra* (Nick, '12), and *Crocodylus* (Gaupp, '05 b; Shiino, '14), I find no suggestion in *Eumeces*. This structure is not mentioned by Gaupp in *Lacerta* and its presence in *Dermochelys* is denied by Nick.

3. Roof of ethmoid region

Owing to the need of external protection, the roof of the nasal capsule or tectum nasi (fig. 1) is very complete as compared with the very imperfect floor of this region. The two main nasal cavities, right and left, are domed over completely by a thin roof of cartilage except for the enormous fenestrae superiores nasi (figs. 1 and 27, *fen.s.na.*) already mentioned. In stage 6

these fenestrae retain approximately the same absolute size, but are relatively smaller than in stage 5. At the anterior and posterior the tectum runs in as a point between the fenestrae of the two sides, but in the middle the two fenestrae are separated only by the free upper edge of the septum. Functionally, the lack of cartilage in this region is compensated by the strong early development of the nasal bones (figs. 1 and 28, *os na.*), which, even in stage 5, form an almost complete roof over the fenestrae. The fenestrae superiores are present in *Lacerta*, although decidedly smaller than in *Eumeces*, and in *Sphenodon*, according to Schauinsland ('00) and Howes and Swinnerton ('01). Ogushi ('11) figures very large fenestrae in adult *Trionyx*, but I find no other record of their presence in turtles, crocodiles, or snakes. Their absence in *Emys* is positively affirmed by Kunkel ('12 b), nor do they appear in the figures of Nick ('12) for *Dermochelys*, *Chelone*, and *Chelydra*, of Parker ('83, reproduced by Gaupp, '05 b) for *Crocodylus*, or of Peyer ('12) for *Vipera*.

In the posterior third of the ethmoid region the tectum is extended uninterruptedly into a pair of great, ear-like, lateral expansions which roof over the extraconchal recesses of the nasal capsule (figs. 1 and 26, *p.ext.*); at the extreme front, also, it forms a projecting lobe on each side, extending a little in advance of the nasal septum (fig. 1). Laterally the tectum goes over, without interruption and without sharp demarcation of any kind, into the lateral wall or *paries nasi*.

Two pairs of small foramina penetrate the tectum *nasi*. The one pair, foramina *apicalia* (fig. 1, *f.ap.*), are located at the sides of the extreme anterior end of the septum; the others, foramina *epiphania* (figs. 1 and 26, *f.ep.*), are located in the neighborhood of the conchal infolding of the nasal wall. These foramina serve, respectively, as exits for the two main branches of the ethmoid ramus of the trigeminus nerve.

4. Lateral wall of ethmoid region

The most striking characteristic of the paries nasi or lateral wall of the ethmoid region in stage 5 of *Eumeces* (fig. 3) is its imperfection as compared with that of *Lacerta*. In the anterior third, especially, the paries is reduced to a narrow margin of the tectum, slightly deflected ventrally. On this deflected margin two projecting lobes may be distinguished—the processus alaris inferior (*pr.al.i.*), at the tip of the snout, and the processus alaris superior (*pr.al.s.*), a little further posterior, or rather dorsal because of the downward bend of the snout. In the notch between these two processes is located the external nostril. The processus alaris superior is far less prominent than in Gaupp's figures and model of *Lacerta*; in *Crocodylus* (Shiino, '14) and *Emys* (Kunkel, '12 b) there are no alar processes; in *Vipera* (Peyer, '12) they are well developed.

Between the anterior and middle thirds of the ethmoid region the paries is notched out rather strongly, just posterior to the superior alar process, broadening again immediately in a projecting lobe which occupies the entire middle third. Here, more than elsewhere, the lateral wall approaches the solum nasi, but, in this stage, it is very far from the fusion in *Lacerta*, on the strength of which Gaupp names a 'zona annularis,' in which the nasal cavity is surrounded by a complete ring or tube of cartilage. The contrast here is due mainly to late chondrification in *Eumeces*; in stage 6 the union is complete, although the connecting cartilage is still histologically very young. In fact, this connection is already suggested in stage 5 by a condensation of the embryonic connective tissue, which, however, hardly deserves the name of procartilage. In the earlier stages there is not even this suggestion of the union. Even in stage 6 of *Eumeces* the term *zona annularis* is hardly justified, for, just where the lateral wall and solum are united, the tectum is largely obliterated by the exceptionally large fenestra superior nasi (fig. 27). The contrast with *Lacerta* is, however, one of degree, not of kind. The presence of the *zona annularis* in *Lacerta* is confirmed by Becker ('03), who describes a similar structure in *Platydictylus*. It is conspicuously developed in the turtles (Nick, '12; Kunkel,

'12 b) and crocodiles (Shiino, '14), in correlation with the general completeness of the ethmoid region; on the other hand, in *Vipera* (Peyer, '12) it is merely suggested in the procartilage stage, and the continuity is soon interrupted.

In the posterior third of the ethmoid region the *paries nasi* is most extensive and, at the same time, most complicated, owing to the formation of a great lateral bulb (fig. 2, *p.ext.*), housing the extraconchal recess of the nasal cavity. This extraconchal part of the nasal capsule, as already noted, extends forward as well as laterally, so that it comes to lie in part at the side of the middle third of the capsule. From this it is separated by the conchal infolding (fig. 2, *co.*) and its anterior opening, the *aditus conchae* (fig. 1, *ad.co.*). The *concha* (fig. 26, *co.*) has the form of an inverted trough, extending obliquely from anterodorsal to posteroventral, and is open, in this stage, not only at the anterior end, but also ventrally throughout its entire length. At its posterior end the trough is terminated by the bending together of its lateral, dorsal, and medial walls. On one side of the specimen modeled this posterior closure is incomplete. Conditions have not materially changed in stage 6, but, in still later stages, it may well be that the *concha* becomes definitely tubular through the closure of the posterior part of its ventral opening, as described in the later development of *Lacerta*. Lateral to the *concha* the *paries nasi* is so modified that it forms not only the lateral wall, but also a roof, a floor, and a partial median wall for the extraconchal recess (fig. 26); in the region of the *aditus conchae*, as may be seen from figures 1 and 2, the section of the extraconchal portion of the nasal capsule would be a closed circle. Behind the *concha* (fig. 25), the *paries nasi* again takes on a simple configuration. A *concha* similar to that of the lizards is described in *Vipera* by Peyer ('12) and *Crocodylus* by Shiino ('14); in the turtles it is rudimentary (Gaupp, '05 b; Nick, '12; Kunkel, '12 b).

In a discussion of the mechanical origin of the *concha*, Gaupp ('00) cites the view of Seydel ('95, '96), who interprets the external nasal gland as an active factor, pushing in the capsular cartilage, and thus separating off the extraconchal recess. Gaupp himself

inclines rather to the view that the active factor is to be found in the growth and expansion of the olfactory epithelium. Thus the extraconchal recess is primary and the concha secondary, the expansion of the nasal chamber being prevented at this point by the passive resistance of the gland. In a later publication ('05 b) he modifies this view, and follows Born ('79, '83), who accords even less of mechanical significance to the gland. The folding of the olfactory epithelium and its limiting cartilage give rise to the conchal invagination, "in die sekundär die glandula nasalis lateralis von vorn her hineinwachst." The development of *Eumeces* speaks strongly for the secondary relation of the gland to the concha. In stage 6 the gland lies, as described by Gaupp, deep in the aditus and the inverted trough of the concha itself. From this position the duct (figs. 27 and 28, *d.l.na.*) is easily followed along the lateral face of the cartilage wall of the zona annularis to enter the nasal cavity through the notch separating the superior alar process from the zona annularis. On the other hand, even as late as stage 5, the apparently solid anlage of the gland has grown backward only to a point slightly in front of the extreme anterior extension of the extraconchal recess, and thus well outside of the aditus conchae. The infolding of the concha is already well established, but the slowly developing gland rudiment is lagging far behind.

In the lateral wall of the recessus extraconchalis of *Lacerta* Gaupp figures an enormous fenestra lateralis nasi, upon which he lays some emphasis—"Auf die grosse Fenestra nasi lateralis sei aber besonders hingewiesen, mit Rücksicht auf die Kieferhöhle der Säuger, deren Entstehungsort in dieser Gegend zu suchen ist" ('00, p. 568). In *Eumeces* there is no suggestion of such a fenestra, the lateral wall being perfectly continuous in this region in stage 5 (figs. 25 and 26), and in all other observed stages in which the cartilage is recognizable; nor is there any weakening of the wall in the later stages to suggest an approaching resorption of cartilage, as in the fenestration of the nasal and inter-orbital septum described above.

Gaupp ('05 b) has himself called attention to the recorded absence of the fenestra lateralis in the crocodile (Parker, '83)

and even in some lizards (Born, '79, who mentions *Scincus*, *Gongylus*, *Lygosoma*, *Eumeces*, *Euprepes*, *Marethia*, *Hinulia*, and *Hemidactylus*); more recently its absence has been confirmed in the crocodile (Shiino, '14) and reported in *Platydictylus* (Beecker, '03), *Vipera* (Peyer, '12), *Dermochelys*, *Chelone*, and *Chelydra* (Nick, '12), and *Emys* (Kunkel, '12 b). The frequency of its absence in the reptilian series suggests the possibility that the fenestra lateralis of *Lacerta* may be nothing more than another area of retarded chondrification like those described in the otic capsule of *Lacerta*, *Emys*, and *Eumeces*—a possibility which gains in significance from the absence of the fenestra in mammalian embryos—mole (Fischer, '01 b), *Echidna* (Gaupp, '08 a), pig (Mead, '09), rabbit (Voit, '09 b), dog (Olmstead, '11). Another explanation is suggested by the conditions in *Sphenodon*. The fenestra lateralis is not recorded by Schauinsland ('00); but, according to Howes and Swinnerton ('01), it makes its appearance in very late embryos. It has also been found in the adult skull of *Trionyx* by Ogushi ('11), although lacking, as noted above, in embryos of other turtles and even in the newly hatched young studied by Nick ('12). A precocious development of the fenestra in *Lacerta* and a retardation in the other forms might well explain the strikingly contradictory conditions. Only a further investigation, particularly of late embryos and adults, can determine the history of the fenestra lateralis and the question of its significance or non-significance.

Just posterior to the concha, the free ventral and posterior margins of the paries nasi meet in an angle of about 90° , marking the point of its greatest extension (fig. 3, *p.tr.*). This is clearly the equivalent of the more extensive *pars triangularis* of *Lacerta*. Near this, but connected in stage 5 only by a mass of procartilage or condensed connective tissue, is an isolated and rather irregular cartilage element (figs. 2 and 3, *pr.max.a.* and *pr.max.p.*), to be homologized with the maxillary processes, anterior and posterior, of *Lacerta*. In stage 6 this mass is continuous with the nasal capsule, as in *Lacerta*, while in the earlier stages the separation is even greater than in stage 5. In stage 5 it is possible to distinguish a rudimentary anterior process (*pr.max.a.*)

and a more highly developed posterior one (*pr.max.p.*). As in *Lacerta*, the anterior process and the main body of the posterior process follow the palatal plate of the maxillary bone (figs. 1 and 25), although not in contact with it. The posterior process also gives rise to a median branch, in the form of a slender rod of cartilage (figs. 2 and 25, *pr.max.p'*), which extends across from the maxillary bone to the palatine (fig. 25, *os pal.*), and then bends sharply to the posterior, lying for some distance in a conspicuous groove in the lateral surface of the palatine bone. In *Lacerta Gaupp* indicates the same structure, but in a degenerate or, possibly, undeveloped condition—

Nahe seinem hinteren Ende (i.e., of the *processus maxillaris posterior*) geht von ihm noch ein besonderes Querstück medialwärts und schiebt sich auf das *Os palatinum* herauf und in der Verlängerung dieses Querstückes finden sich noch einige variable kleine Knorpelinseln auf dem *Os palatinum* (Fig. 3). ('00, pp. 483 and 484.)

Zwischen ihm (i.e., the *processus maxillaris posterior*) und dem hinteren Ende der *Cart. paraseptalis* bilden sich vorübergehend auf der Dorsalfäche des *Palatinum* kleine Knorpelinseln, die wahrscheinlich Andeutungen dafür sind, dass das *Planum antorbitale* mit seinem ventralen Rande früher dem *Palatinum* aufruhte (Fig. 384). An ihrer Stelle wurde auch einmal ein mit der *Cart. paraseptalis* zusammenhängender Knorpelfortsatz gefunden. ('05 b, p. 766.)

There is nothing in *Eumeces* to suggest any such connection of this palatine extension of the *processus maxillaris posterior* with the paraseptal cartilage and the *planum antorbitale*. It seems more probable that the continuous rod of *Eumeces* and the cartilage fragments ('Inseln') figured by Gaupp belong to the maxillary process itself, which is homologized as forming the anterior part of an originally continuous palato-pterygo-quadrate bar of cartilage (see also p. 199). Gaupp gives no figure of the "mit der *Cart. paraseptalis* zusammenhängender Knorpelfortsatz;" from the very brief description I am unable to estimate its significance in this connection. A similarly close relation of the posterior maxillary process with the maxillary and palatine bones is emphasized by Fuchs ('09) in *Sphenodon* and the *Geckonidae*; the presence of the process in *Sphenodon* was earlier recorded by Schauinsland ('00). Among the turtles it is present in *Dermochelys* (Nick, '12), but

lacking in *Chelonia* and *Chelydra* (Nick, '12) and *Emys* (Kunkel, '12 b). It is also absent in *Vipera* (Peyer, '12) and *Crocodylus* (Gaupp, '05 b; Shiino, '14).

The *paries nasi* has been described above as ending posteriorly in a free margin; this is only partially true. From the *pars triangularis* this margin curves upward and backward and toward the median plane; thus the lateral wall, rapidly narrowing, is transformed into a posterior wall of the nasal capsule, the *planum antorbitale* (figs. 1, 2, and 3, *pl.ant.*). The narrowing of the wall occurs mainly at the lower edge, so that the free upper edge of the *planum antorbitale* lies just under the sphenethmoid cartilage and only very slightly below the upper edge of the nasal septum (fig. 3). The *planum antorbitale* is a nearly vertical plate, and extends almost transversely, although its inner end is very slightly posterior to the outer. In the neighborhood of the nasal septum its median end bends suddenly downward, then as suddenly forward, and goes over into the paraseptal cartilage (fig. 2, *c.par.*), to be described more fully in connection with the floor of the ethmoid region. It should be noted that the *planum antorbitale* remains entirely free from the septum, as in most reptiles—*Sphenodon* (Schauinsland, '00), *Chrysemys* (Gaupp, '05 b), *Emys* (Gaupp, '05 b; Kunkel, '12 b), *Chelydra*, *Chelone*, and *Dermochelys* (Nick, '12). In *Dermochelys* and *Chelonia* there is contact, but no fusion of the cartilages. Only in *Crocodylus* is a fusion reported, and here the data are contradictory. Gaupp ('05 b) reports the *planum antorbitale* as free, while Shiino ('14) describes it as uniting solidly with the septum. In *Vipera* (Peyer, '12) the entire *planum antorbitale* is lacking. In the *Mammalia* the *planum antorbitale* may be either free or attached, the latter condition being held as secondary by Gaupp, who stresses the freedom of the *planum antorbitale* in their reptilian ancestors as affording the opportunity for a shifting of the position of the posterior limit of the nasal capsule in the *Mammalia*—

Die Nasenkapsel der Säuger leitet sich von einer Kapselform ab, bei der, wie z.B. bei *Rhynchocephalen* und *Sauriern*, die Hinterwand (d.i. die beiderseitigen *Plana antorbitalia*) sowie die *Cartilago para-*

septalis nur in loser Verbindung mit dem Septum standen. Indem auf der Grundlage eines solchen Zustandes die Nasenhöhle bei den Säugern eine grössere Entfaltung erfuhr, konnte die Hinterwand nach hinten hin vorgeschoben werden, wodurch ein Theil des früheren Septum interorbitale (der reptilischen Säuger-Ascendenten) in intranasale Lage kam. ('08 a, p. 776.)

5. Floor of ethmoid region

The cartilaginous floor of the nose (fig. 2), as already noted, is extremely incomplete, and the nasal capsule would be largely open ventrally except for the very perfect adaptation of the vomer (fig. 2, *os vo.*) to supply the deficiency of cartilage. Through almost its entire length the ventral edge of the nasal septum is free from all other cartilages; only at the extreme anterior end is it fused with an insignificant horizontal plate of cartilage, the *solum nasi*, in the narrow sense, or *lamina transversalis anterior*. Toward the posterior this floor separates almost immediately from the septum and forms, on each side, a little saucer of cartilage, the capsule of Jacobson's organ (fig. 2, *caps.jac.*), which supports the anterior half of Jacobson's organ. The hinder margin of this saucer is divided into three rather definite lobes. The innermost lobe has a thin edge and terminates further forward than the others; lateral to this the cartilage is swollen into a rounded knob, which pushes up into Jacobson's organ, forming its *concha* (figs. 2 and 28, *co.jac.*, more conspicuous a few sections further back than fig. 28); the outermost lobe (fig. 2, *c.ect.*) is also considerably thickened and extends furthest to the posterior, although to no such distance as the corresponding element, *cartilago ectochoanalis*, in *Lacerta*.

In stage 5 this capsule of Jacobson's organ has no further connections, but in a later stage (fig. 27) its lateral margin is continuous with the lateral wall of the nasal capsule, to form the incomplete *zona annularis* already mentioned. In *Lacerta* its median lobe extends back continuously into the paraseptal cartilage, a thin strip of cartilage which lies close alongside the lower edge of the septum, but without contact, and is continuous posteriorly with the *planum antorbitale*. Alike in *Eumeces* and

Lacerta, it is set obliquely, with one face turned upward and outward, the other inward and downward (figs. 25 and 26, *c.par.*). In Eumeces (fig. 2) the paraseptal cartilage forms independently of the capsule of Jacobson's organ, and is widely separated from it in the earlier embryos; during development the gap is gradually lessened and, in stage 6, is entirely closed on one side, while the interruption on the other side is very slight. Thus conditions become essentially as described for Lacerta. Kunkel ('12 b) notes that the union of the paraseptals with the planum antorbitale occurs at a very late stage in the development of Emys. In Eumeces both cartilages, especially the planum antorbitale, are of relatively late development and difficult to distinguish in younger embryos, but there is no evidence for an original separation and later fusion.

The forward half of Jacobson's organ, as stated, is supported by the cartilaginous capsule; behind this the organ has no cartilaginous support. But this deficiency is supplied by the large trough-like vomer (fig. 2, *os vo.*), which underlies the rudimentary solum nasi (figs. 27 and 28), and, behind it (figs. 25 and 26), takes its place as a support for Jacobson's organ and as a floor for the whole capsule. The vomer extends back almost to the planum antorbitale, where its place is taken by the palatine (fig. 2, *os pal.*). Jacobson's organ is also roofed over and separated from the nasal cavity proper by another membrane bone, the septomaxillary (figs. 27 and 28, *os s-max.*), which forms an inverted saucer with its median edge supported by the septum, its lateral edge by the solum and the maxillary bone. In length it is practically coextensive with Jacobson's organ, to which its functional relation is very apparent.

The floor of the extraconchal recess is really a part of the complexly folded paries nasi, and has been noted already in that connection.

The reptiles show a wide range of variation in the nasal floor as in other parts of the ethmoid region. The floor is highly developed in Crocodilus (Gaupp, '05 b; Shiino, '14) and the turtles (Nick, '12; Kunkel, '12 b), although exceptionally variable in the latter group. In snakes it is very rudimentary. In the

cartilage stage of *Vipera* Peyer ('12) describes the floor as consisting of practically nothing beyond an isolated shell for the support of Jacobson's organ, although he recognizes procartilage connections with the septum and the lateral wall; Born ('83) has recorded a somewhat less rudimentary condition in *Tropidonotus* and *Pelias*. The paraseptal cartilages are generally present throughout the group, but lacking in snakes (Peyer, '12). They are interpreted by Gaupp ('06), following Seydel ('96), as mere detached portions of the floor; before chondrification Gaupp ('05 b) reports them continuous with the septum in the lizards. Their separation from the septum is simply one item in the general process, already discussed in connection with the planum antorbitale, through which the posterior end of the nasal capsule becomes free for its change of position in the mammals.

6. Nerve foramina of ethmoid region

Lastly, a brief account of those openings which serve for the entrance or exit of nerves. Of these nerves there are but two which need occupy our attention—the olfactory (together with the olfactory lobe) and the first, or ophthalmic, ramus of the trigeminal—renamed the ramus ethmoidalis on entrance to the nasal capsule.

The olfactory fenestra (fig. 1, *fen.ol.*), divided into right and left halves by the septum nasale, is an enormous kite-shaped opening with rounded corners and lies in an almost horizontal plane. The lateral angles, formed by the union of the sphenethmoid cartilages with the tectum nasi, are approximately right angles; the anterior angle, formed by the posterior margin of the tectum, is obtuse, or, rather, is reduced to an almost semi-circular curve; the posterior angle, formed by the convergence and union of the sphenethmoid cartilages in the solum supra-septale, is decidedly acute. In the posterior part of the opening lie the long olfactory lobes, from which a very large number of nerve bundles extend into the nasal capsule (fig. 25, *n.I.* and *n.I'*). Only a few of the bundles are indicated by the letters, the others are easily recognized. One pair of these bundles (*n.I.*) are marked out by their very large size and their more

posterior origin from the olfactory lobes. They extend, with little or no branching, into the forward part of the nasal capsule, where they break up very suddenly into numerous small branches leading into the nasal epithelium. They correspond unquestionably to the 'olfactory nerves' as modeled by Turner ('14) from the same sections, as figured by Watkinson ('06) for adult *Varanus*, and as described in the general texts; they are apparently the 'vomeronasal nerves' as distinguished by McCotter ('12, '17). The details of their distribution and their relation to the smaller nerves have not been followed out, but seem to agree with the observations of McCotter, particularly for the *Mammalia* ('12).

Leading from each orbit into the nasal cavity, and separated from the fenestra olfactoria only by the sphenethmoid cartilage, is an irregular slit, the fissura orbitonasalis (fig. 3, *fis.o-na.*), through which the ethmoid ramus of the trigeminus gains access to the nasal chamber. Entering this chamber ventral to the olfactory lobe, it makes its way to a position above and to the side of the group of olfactory nerve bundles; here it divides into two branches (fig. 25, *n.V.eth.e.* and *n.V.eth.i.*). The external ramus passes on with little change of direction, emerges through the small foramen epiphaniale noted above the aditus conchae (fig. 26, *n.V.eth.e.* and *f.ep.*), and supplies the region of the glandula lateralis nasi. The ramus interna sweeps across the nasal cavity, dorsal to the olfactory nerve bundles, and reaches the septum; it then descends rapidly along the septum to the level of the septomaxillary bone, and follows the median margin of this bone forward, finally emerging through the foramen apicale at the tip of the snout (fig. 1, *e.ap.*). Its course is easily traced by comparing its position (*n.V.eth.i.*) in figures 25, 26, and 28.

The arrangement of these openings and the distribution of the nerves is essentially alike in *Lacerta* and *Eumeces*; in other reptiles some rather striking variations are recorded. Thus, the sphenethmoid cartilage is lacking in *Crocodylus* (Shiino, '14) and *Vipera* (Peyer, '12), and the fenestra olfactoria and fissura orbitonasalis become perfectly confluent. Among the turtles Nick ('12) has reported very marked differences in this respect;

in Chelydra the sphenethmoid cartilage is entirely lacking, while in Dermochelys it is perfectly developed; in Chelonia it may be well developed or rudimentary. Emys (Kunkel, '12 b) is like Dermochelys in the presence of this dividing cartilage between the fenestra olfactoria and fissura orbitonasalis. When the cartilage is lacking, the ethmoid ramus of the trigeminus ordinarily enters the nasal capsule in company with the olfactorius; in the crocodile a very aberrant course is described by Shiino ('14). Here the ethmoid ramus passes not into the nasal cavity, but directly to the free upper surface of the nasal tectum, where it lies in the 'sulcus terminalis,' corresponding to the aditus conchae. Another unusual arrangement is described by Nick ('12) in Dermochelys—the presence of a foramen for the external ethmoid ramus (for the internal ramus as well in one specimen) independent of the fissura orbitonasalis, which is perfectly enclosed in this form. The foramen epiphaniale, for the exit of the external ramus, seems to be of almost universal occurrence; it is specifically mentioned for Sphenodon (Schauinsland, '00), Vipera (Peyer, '12), Chelone and Chelydra (Nick, '12), and Emys (Kunkel, '12 b). In the adult of Trionyx a foramen in the exact position of the foramen epiphaniale is figured by Ogushi ('11), who, however, identifies it as the 'foramen ductus glandulae nasalis.' Only in Crocodilus (Shimo, '14) is the absence of the foramen epiphaniale affirmed—a natural correlative of the remarkable course of the ethmoid nerve in this form. Data are scanty concerning the foramen apicale. In Crocodilus its presence is noted by Shiino ('14); in Emys it is reported lacking by Kunkel ('12 b), who, however, gives no detailed account of the course of the ramus internus.

8. MANDIBULAR ARCH

1. General description

Various parts of this structure have been more or less fully described in preceding pages; it is desirable now to collect these scattered data and to give a more consecutive account of the entire arch. The cartilaginous mandibular arch is composed

of dorsal and ventral divisions. The ventral, throughout the vertebrate series, consists of Meckel's cartilage, the cartilage of the lower jaw; the dorsal consists of a more or less continuous bar of cartilage, the palato-pterygo-quadrato cartilage, furnishing the primitive supporting apparatus or suspensorium for the lower jaw.

In early stages of *Sphenodon* (Howes and Swinnerton, '01), *Lacerta* (Cords, '09), *Testudo* (Bender, '12), and *Crocodylus* (Shiino, '14) the dorsal and ventral divisions of this arch are described as represented by a single continuous mass of blastema. This condition is probably general, but the articulation is already well developed in the earliest observed stages of *Eumeces*.

2. Dorsal division—palato-pterygo-quadrato cartilage

In the *Anura* (Gaupp, '93; Fuchs, '09) there is a continuous bar of cartilage extending from the nasal capsule to the otic capsule; in the reptiles this has suffered a varying degree of degeneration. In *Eumeces* only the posterior portion is highly developed. This appears as a large and well-formed quadrato cartilage (fig. 3, *quad.*), extending forward and somewhat downward from the neighborhood of the crista parotica of the otic capsule to the articulation of the lower jaw. Its general form is that of a thick and rather narrow plate, so twisted in its course that its upper end approaches a parasagittal plane (fig. 11), while its lower articular extremity is more nearly horizontal (fig. 17). The articular surface is saddle-shaped—convex in parasagittal, concave in horizontal section. The lateral portion of the articular surface is extended as a conspicuous process, giving an oblique form to this end of the cartilage (fig. 2). In its middle third the dorsal edge of the quadrato is raised into a fine projecting crest with slightly thickened and outwardly rolled margin (figs. 3, 10, 12, and 16). In stage 6 this projecting edge is much more strikingly recurved, so that it comes to form an almost semicircular frame for the support of the dorsal side of the developing tympanic membrane.

In *Lacerta* this crest is much less developed than in *Eumeces*; in *Emys* (Kunkel, '12 b) and *Testudo* (Bender, '12) it is enor-

mously exaggerated. In *Emys* the upper part of the quadrate is described as forming a 'hollow cone.' The entire quadrate is very massive, and located more posteriorly than in the lizards, so that its posterior margin must be deeply excised for the stalk of the columella auris. In *Testudo*, on ossification, it actually forms a canal around the columella.

In stage 5 of *Eumeces* the quadrate is movably attached to the otic capsule by way of the fused processus paroticus and crista parotica. The more complete cartilaginous union with the processus paroticus in earlier stages has been described (p. 156), together with Versluys's interpretation of the processus paroticus as the processus dorsalis of the columella auris. In the turtles, according to Gaupp ('05 b) and Bender ('12), and in the crocodile, according to Gaupp ('05 b), the quadrate is free in early stages, although soon fusing with the otic capsule. I have no opinion to express concerning the bearing of these observations upon the vexed question of the relation of the streptostylic and monimostylic types of skull structure.

Anterior to the quadrate (and entirely separate from it in stage 5 of *Eumeces*) is located a group of cartilages which stand in intimate relation to the pterygoid bone and the basipterygoid process—epipterygoid, processus pterygoideus, and articular cartilage. These have been mentioned in connection with the orbitotemporal region (p. 172). In harmony with the view of Parker ('80, '83), they are explained by Gaupp ('91 a, '91 b, and later papers) as belonging to the first visceral arch. Thus the epipterygoid (fig. 3, *epipt.*) is homologized with the processus ascendens of the amphibian quadrate, while the processus pterygoideus is represented by the horizontal rod of cartilage (figs. 1 and 3, *pr.pt.*) extending forward from the foot of the epipterygoid in a shallow groove of the pterygoid bone.

That the nodule of cartilage (fig. 1, *pr.pt'*) in connection with the lateral anterior branch of the pterygoid bone is merely an isolated fragment of this rod can hardly be questioned, in view of Gaupp's observation of a continuous extension of the pterygoid process into this position in *Lacerta*. A similar discontinuous fragment of the pterygoid process is described by Schauins-

land ('03) in *Sphenodon*; and in an embryo of *Lacerta vivipara* Gaupp ('91 b) records the processus pterygoideus as composed of three more or less isolated fragments. That the processus pterygoideus and the epipterygoid are confluent in earlier stages of *Eumeces* has been noted. In stage 5 these structures are rather widely separated from the quadrate; but in stage 2 a band of young cartilage or procartilage extends backward along the pterygoid bone and connects with the articular extremity of the quadrate. A similar condition is described by Gaupp ('91 a, '91 b, '05 b) in *Lacerta*. Thus the processus ascendens (epipterygoid) and processus pterygoideus are brought into their normal relations to the body of the quadrate. This homology gains in force from the much more complete and more persistent connection with the quadrate in the lizards, *Mabuia*, *Zonurus*, and *Eremias* (Broom, '03), the crocodile (Gaupp, '05 b, citing and confirming Parker, '83; Shiino, '14), *Sphenodon* (Schauinsland, '00; Howes and Swinnerton, '01; Fuchs, '12), and the turtles, *Chelydra* and *Dermochelys*, (Nick, '12), *Chelone* (Parker, '80; Nick, '12; Fuchs, '12), and *Emys* (Filatoff, '06; Noack, '07, if, as is probable, the 'prootic' of his figures represents the combined pterygoid and ascending processes; Kunkel, '11, '12 b). The separation of the processes from the body of the quadrate in the lizards Fürbringer ('04) correlates with the free motion of the quadrate in this group; in this connection Gaupp's record ('05 b) of the absence of processes in the very simple quadrate of the snake is significant.

While the quadrate character of the epipterygoid and processus pterygoideus is almost certainly established, the relations of the articular cartilage or meniscus pterygoideus (fig. 1, *c.art.*), lying between the processus basipterygoideus and the pterygoid bone, are far less clear. This, also, is held by Gaupp ('91 a and later papers) to belong to the dorsal part of the mandibular arch; this view is accepted as probable by Fuchs ('09). In early stages of *Eumeces* the articular cartilage is clearly confluent with the common rudiment of epipterygoid and processus pterygoideus, which suggests a genetic relationship; on the other hand, it is also continuous, in these early stages, with the processus basi-

pterygoideus, which complicates the problem. Both of these connections are noted by Gaupp in *Lacerta*. The presence of the meniscus is recorded in *Sphenodon* by Gaupp ('05 b) and in *Emys* by Fuchs ('12) and Kunkel ('12 b). In *Emys*, according to Kunkel, it is rudimentary and soon disappears; the disappearance is interpreted as probably due to fusion with the 'crista basipterygoidea.' I find no mention of the meniscus in the crocodiles.

The forward part of the palato-pterygo-quadrato cartilage is represented in *Eumeces* by the posterior maxillary process (fig. 3, *pr.max.p.*) and its posterior palatine extension (*pr.max.p'*), already described in connection with the ethmoid region (p. 191). In *Lacerta* the posterior extension of the process is reduced to a series of isolated cartilage islands. Between the processus maxillaris posterior and the processus pterygoideus, the palato-pterygo-quadrato cartilage, continuous in the *Anura*, is interrupted in the *Reptilia* by a varying degree of degeneration. In *Eumeces* (fig. 3) and *Lacerta* the gap is very considerable, and in *Vipera* (Peyer, '12) the degeneration has reached its extreme in the loss of the entire structure anterior to the quadrato. On the other hand, a more primitive condition is retained in *Sphenodon* (Schauinsland, '03; Gaupp, '05 b; Fuchs, '09), in which the two processes are much more closely approximated—a condition only slightly removed from the continuity of the anuran.

3. Ventral division—Meckel's cartilage

Meckel's cartilage (fig. 4), forming the ventral division of the mandibular arch, is essentially as in *Lacerta*. The surface for articulation with the quadrato is slightly saddle-shaped—concave in the parasagittal plane and convex in the horizontal. Posterior to the joint, the cartilage is extended into a long retroarticular process (fig. 4, *pr.ret.*), which is strongly compressed in a nearly dorsoventral direction (figs. 17 and 18). In front of the articulation, the cartilage is almost circular in cross-section (figs. 20, 21, and 24, *c.meck.*), except at the extreme anterior end, near the symphysis, where it becomes laterally compressed (fig. 28). The two rami meet in an acute angle, lie in contact for some

distance, and finally fuse continuously in the symphysis; no independent cartilage is found in the angle of the symphysis. This description applies to all stages studied.

The general form of Meckel's cartilage in the reptiles is subject to marked variation in correlation with the varying function of the mouth. Compare, for example, its heavy structure in Emys (Kunkel, '12 b, fig. 27) with its delicate form in Eumeces (fig. 4). In the former, the ratio of its diameter, half-way between articulation and symphysis, to its length, from articulation to symphysis, is about 1 to 7; in Eumeces the corresponding ratio is approximately 1 to 28. Two further points call for brief mention here—the reduction of the processus retroarticularis in turtles, and the structure of the symphysis. In Testudo Bender ('12) describes the processus retroarticularis as entirely lacking, although his figure 23 seems to indicate its presence in rudimentary condition; in Emys, also, Kunkel ('12 b) describes the process as very small, especially in later embryos. As to the symphysis, the data show much of diversity and somewhat of contradiction. An independent 'basimandibulare' in the angle of the symphysis is recorded by Parker in Crocodilus ('83) and Chelone ('80). Gaupp ('05 b) also describes a 'ziemlich selbstständiges Knorpelstück' in this position in turtles. In Emys, according to Kunkel ('12 b), the angle is filled by a mass of cartilage, but this is interpreted as belonging to the rami themselves, and not to an independent cartilage in the sense of Parker. Bender ('12) denies the presence of a connecting piece in Testudo, and Shiino ('14) in the crocodile. In the snake (Peyer, '12) the rami are in contact at one stage of the development, but there is no fusion.

9. HYOID AND BRANCHIAL ARCHES

The question of the relation of the columella auris to the hyoid arch, already discussed in detail (p. 160 et seq.), will not be considered here.

The balance of the hyobranchial apparatus (fig. 4) agrees essentially with the account of Lacerta as given by Gaupp ('05 a, '05 b). The body of the hyoid consists of an insignificant tri-

angular plate of cartilage (*hy.cor.*), whose anterior angle is drawn out into a long and slender cylindrical rod, the processus entoglossus or processus lingualis (*pr.lin.*). The latter name is to be preferred, as emphasizing the homology of this structure in the lizards with the processus lingualis of the turtles and not the independent 'entoglossum' described by Gaupp ('05 b) and Bender ('12). There is no suggestion in *Eumeces* of the paired origin of the processus lingualis noted in *Emys* by Fuchs ('07 b) and Kunkel ('12 b). The body of the hyoid is located a little below the plane of the lower jaw; the processus lingualis terminates slightly above this plane. From each posterolateral corner of the body of the hyoid, three rods of cartilage diverge. The most anterior of these is the hyoid arch (*hy.ar.*), the others (*br.1* and *br.2*) are homologized with the first and second branchial arches of the gill-breathing vertebrates. The hyoid arch extends forward for a short distance; then, doubling upon itself in an acute angle, it stretches backward, outward, and upward, to terminate in close proximity to the insertion plate of the columella auris, with which it is continuous in earlier stages. The first branchial arch follows a course approximately parallel to that of the recurved portion of the hyoid arch and terminates slightly posterior and ventral to its termination. The second branchial arch is short; it extends backward in the plane of the body of the hyoid.

In addition to these connected structures, a pair of unattached cartilages (*br.2'*) demands mention. Each begins in close proximity to the otic capsule, just posterior to the cochlear prominence, extends downward just median to the hyoid and first branchial arches, and terminates directly in line with the second branchial arch, but somewhat posterior to it. A similar isolated fragment in *Lacerta* is interpreted by Gaupp ('05 a) as probably belonging to the second branchial arch; in one specimen of *Lacerta vivipara* the two are almost in contact. On the other hand, Cope ('92), who notes the presence of this cartilage in adults of a number of species of the *Lacertidae*, *Xantusidae*, and *Scincidae* (including *Eumeces quinquelineatus*), interprets it as a 'free epibranchial' belonging to the first branchial arch, and Cords ('09) reports a

direct connection with the first branchial in one embryonic stage of *Lacerta vivipara*. This observation by Cords would seem to be conclusive, but the study of early stages of *Eumeces* leads me to question the character of the reported connection. Here also, in stages 1 and 2, the element in question is in contact with the first branchial at one point, and the two procartilage rudiments are hard to distinguish from one another. A careful study shows that this contact is a lateral one, and that the ends of the two structures are free in these embryos, as in stage 5. The contact and seeming union are probably only secondary. It is significant, in this connection, that Cords reports the problematic cartilage as arising from the first branchial at a sharp angle ('mit einer scharfen Knickung'), and adds: "Medial von der Knickungsstelle findet sich aus dem Vorknorpelstrang hervorgehend ein hakenförmiger kleiner Fortsatz." This description suggests strongly a condition like that observed in early stages of *Eumeces*. The position and course of this isolated fragment, alike in early and late embryos, suggest strongly a relation to the second branchial arch and make the derivation from the first branchial decidedly difficult. In the adult (Cope, fig. 29) the positions have shifted considerably, but the embryonic conditions are clearly more pertinent. A further study of the early development of this cartilage, in connection with that of the gill clefts, will be needed for a final determination of its homologies, but, for the present, I hold, with Gaupp, that the derivation from the second branchial arch is the more probable.

Earlier and later stages show no conspicuous modifications of the hyobranchial apparatus. The arches are relatively longer in later stages. In stage 6 there is a striking flattening of the distal limb of the hyoid arch, just beyond the point of bending; in stage 5 of *Eumeces* this flattening is merely suggested, but it is conspicuous in Gaupp's figure of *Lacerta* ('05 b). During the development there is also a progressive tendency toward the breaking up of an originally continuous anlage into separate cartilaginous elements. In stage 5 there is a definite threefold division of the cartilage where the hyoid and first branchial arches meet one another and the body of the hyoid. This is shown in

histological differentiation of the cartilage and in slight external grooves (fig. 4), but the three cartilages are in very close contact, probably in actual continuity. Stages 4 and 6 are identical in this respect with stage 5, but in stage 3 the two arches are perfectly confluent with each other at this point, although divided from the body of the hyoid; in stage 2 the confluence includes the latter cartilage also, and there is an uninterrupted anlage for the entire hyobranchial apparatus, aside, of course, from the isolated 'epibranchial' just discussed. In no observed stage is there any suggestion of segmentation at the connection of the second branchial arch with the hyoid body, such as that in *Emys* (Fuchs, '07 b; Kunkel, '12 b), nor at the bend of the hyoid arch, as figured by Osawa ('98, reproduced by Gaupp, '05 b) in *Sphenodon*. This progressive articulation of the hyoid and branchial anlagen has been noted by Gaupp ('05 b) in *Lacerta* and by Fuchs ('07 b) and Kunkel ('12 b) in *Emys*.

10. SUMMARY

The chondrocranium of *Eumeces* agrees closely with that of *Lacerta* in the main outlines of structure and development; in the following summary statement special emphasis is laid upon points of distinction, some of which are rather conspicuous and not without significance.

1. Basal plate interrupted by large fenestra basicranialis posterior.
2. Conspicuous intercondyloid incisure, bounded laterally and ventrally by large crescentic condylar surface—a type leading equally well to sauropsidan monocondyly or mammalian dicondyly.
3. Cartilage of dens epistrophei seemingly confluent with that of basal plate.
4. Notochord mainly dorsal to basal plate, but slightly embedded in latter posteriorly, especially in earlier stages; anterior extremity in regressive development, in earlier stages reaching and penetrating cartilage of crista sellaris, in later stages terminating freely in fenestra basicranialis posterior.

5. Hypoglossus foramina two or three, sometimes asymmetrical on two sides; distinct nerve roots more numerous than foramina—at least five in some specimens.

6. Course of abducens nerve (except for one asymmetrical individual variant with double perforation of basal plate) through tunnel in anterior edge of basal plate, as in *Lacerta*; real point of exit from cavum cranii not this tunnel, but fenestra metoptica.

7. Evidence as to nature of tectum posterius inconclusive; tectum apparently, at least in part, of occipital origin.

8. Cochlear portion of otic capsule greatly exaggerated in *Eumeces* as compared with *Lacerta*; outer contours and inner structure of capsule otherwise essentially similar in both forms.

9. Facialis foramen displaced to intercapsular position, as consequence of exaggeration of cochlear portion of capsule, and 'prefacial' commissure rendered rather 'suprafacial'—conditions strikingly suggestive of *Mammalia* and strongly favoring Gaupp's view of progressive invasion (*Amphibia-Reptilia-Mammalia*) of basal plate by cochlea.

10. Fissura metotica less extended dorsally than in *Lacerta*, and more completely divided, by contact of posterior ampullar prominence and basal plate in region of anterior hypoglossus foramen, into recessus scalae tympani and foramen jugulare.

11. Connective tissue of recessus scalae tympani forming more definite closing membrane for fenestra cochleae, especially in stage 6, than in *Lacerta*; and outer portion of this membrane, corresponding to filling of lateral aperture of recessus in *Lacerta* and early embryos of *Eumeces*, more closely homologous with mammalian membrana tympanica secundaria than recognized by Gaupp.

12. Course of glossopharyngeus nerve in some specimens of *Eumeces*, as in *Lacerta*, typically 'extracapsular'—through recessus scalae tympani; in other specimens of *Eumeces* 'intracapsular'—entering otic capsule through independent foramen in cartilage of median wall; exit in *Eumeces* always through connective tissue of fenestra cochleae and lateral aperture of recessus rather than through cartilage of lateral wall as in certain reptiles. Variation in *Eumeces* not dependent upon age. Seeming contradiction

of 'intracapsular' and 'extracapsular' courses, here and in Reptilia generally, to be harmonized through interpretation of connective tissue of recessus as integral part of otic capsule, differences then depending upon degree of chondrification of capsular wall and relative size of fenestra cochleae.

13. Crista parotica and processus paroticus perfectly confluent in later and more distinct in earlier stages, suggesting independent origin and secondary union. No connection of processus paroticus with stalk of columella auris, but conspicuous tendinous connection with insertion plate. Only negative evidence as regards Versluys's interpretation of processus paroticus as detached processus dorsalis of columella auris.

14. Columella auris conforming essentially to schema of Versluys. Processus internus moderately developed, but not connected with quadrate as in *Lacerta*; processus dorsalis lacking unless represented by processus paroticus; cruciform appearance of insertion plate, so striking in *Lacerta*, destroyed by relatively slight development of processus accessorius posterior and absence of processus accessorius anterior.

15. Relations of chorda tympani to columella auris as in *Lacerta*.

16. Following offered as tentative interpretation of columella. Embryonic connective tissue extending from hyoid arch to otic capsule endowed, throughout its entire extent, with potentiality of cartilage formation. Columella developed in this connective tissue, in connection, on the one hand, with hyoid, on other, with otic capsule, later separating from both. Thus hyostapes genetically related to hyoid and otostapes to otic capsule; entire columella none the less a genetic unit.

17. Complete but gradual fusion of trabeculae in thickened ventral margin of interorbital septum. No other evidence for paired structure of septum.

18. Fenestration of interorbital septum, progressive from earlier to later stages, interpreted as final result of forces originally leading to formation of septum, especially pressure of enlarging eyeballs.

19. Development and relations of processus basipterygoideus essentially as in *Lacerta*, and consistent with Gaupp's interpretation as homologue, wholly or in part, of mammalian alisphenoid.

20. Lateral wall of temporal region in no single stage of *Eumeces* so complete as in Gaupp's figures of *Lacerta*, but all elements and fenestrae of latter recognizable in composite of early and late stages of *Eumeces*. Most cartilages of this region in marked retrogression in stage 5, but taenia marginalis in progression, and just effecting union with otic capsule and solum supra-septale. Fenestra prootica subdivided, in early embryos of *Eumeces*, by additional cartilage elements not noted in *Lacerta*.

21. Solum supra-septale clearly a paired structure, its two halves uniting with septum rather than with one another.

22. Ethmoid cartilages relatively late in their development.

23. Septum nasale dorsally exposed in middle of double fenestra olfactoria, between extraordinarily large fenestrae superiores nasi, and, possibly, between foramina apicalia. Ventral edge of septum considerably thickened, and another less marked thickening in middle height, associated with os septomaxillare. No prenasal extension of septum.

24. Progressive fenestration of septum nasale similar to that of septum interorbitale.

25. Foramina apicalia and foramina epiphania, as in *Lacerta*, serving for exit, respectively, of internal and external branches of ethmoid ramus of trigeminal nerves.

26. Concha and extraconchal portion of nasal capsule as in *Lacerta* except for absence, in all stages studied, of fenestra lateralis nasi. Interpretation of latter not clear—perhaps mere area of retarded chondrification, perhaps precocious development in *Lacerta* and to be sought in later stages of *Eumeces* than those available.

27. Lateral nasal gland not mechanical cause of conchal infolding, but secondarily associated with it in later stages.

28. Superior and inferior alar processes as in *Lacerta*, but less conspicuous.

29. Anterior maxillary process insignificant, posterior highly developed, extending across from os maxillare to os palatinum.

30. Solum nasi, in strict sense, greatly reduced; originally free, and first united with lateral wall in stage 6, forming nearest equivalent of zona annularis of *Lacerta*—incomplete even in this stage owing to dorsal interruption of enormous fenestra superior nasi

31. Posterior edge of rudimentary solum nasi divided into three lobes—lateral, cartilago ectochoanalis; intermediate, thickened to form concha of Jacobson's organ; median, continuous, in stage 6, with paraseptal cartilage. Paraseptal cartilage continuous posteriorly with planum antorbitale; but planum antorbitale and paraseptal cartilage free, in all stages, from septum.

32. Fenestra olfactoria and fissura orbitonasalis separated by sphenethmoid cartilage.

33. Continuous palato-ptyergo-quadrata ramus of amphibian mandibular arch represented in stage 5 of *Eumeces* by the following distinct elements: maxillary processes, including palatine extension of posterior process; processus pterygoideus, including occasional isolated anterior fragment; epiptyergoid (= processus ascendens of quadrata); quadrata; probably, also, articular cartilage of pterygoid. Processus pterygoideus and epiptyergoid continuous with quadrata in earlier stages; apparently also continuous, through articular cartilage, with processus basipterygoideus.

34. Meckel's cartilage very slender, and processus retroarticularis conspicuously developed. No independent 'basimandibular' element in symphysis.

35. Body of hyoid extended into long and slender processus lingualis; hyoid arch and first and second branchial arches well developed. Pair of unattached cartilages tentatively interpreted as isolated fragments of second branchial arches.

36. Development of hyobranchial apparatus marked by progressive division of originally continuous mass into somewhat distinct elements; correlated with this the early separation of hyoid arch and columella auris.

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PLATES

EXPLANATION OF FIGURES

Figures 1 to 8 were drawn by Miss Mabel Hedge from wax-plate models. The length of the model represented in figures 1, 2, and 3 was exaggerated about 10 per cent in the modeling, so that the skull appears abnormally long and narrow. In the other models the exaggeration is so slight as to be negligible.

Figures 9 to 33 are by the author; all except the last two were drawn with the camera lucida or projection apparatus.

The magnifications of figures from models are expressed in relation to the actual specimens, thus corresponding with those of figures of sections. Dimensions and relative ages of the different 'stages' are recorded in the text, page 122

The relative positions of sections figured are indicated by parenthetical numbers—the numbers of the sections in their respective series. Each series is numbered from anterior to posterior; that for stage 5 contains 358 sections, that for stage 6 contains 464 sections. All sections are 15μ in thickness.

The following abbreviations are employed:

ABBREVIATIONS

<i>ad.co.</i> , aditus conchae	<i>com.pr-f.</i> , prefacial basicapsular commissure
<i>b.pl.</i> , basal plate	<i>con.</i> , occipital condyle
<i>b.pl'</i> , extension of basal plate lateral to otic capsule	<i>cr.par.</i> , crista parotica
<i>br 1</i> , first branchial arch	<i>cr.sel.</i> , crista sellaris
<i>br.2</i> , second branchial arch	<i>d.l.na.</i> , duct of lateral nasal gland
<i>br.2'</i> , isolated fragment of second (?) branchial arch	<i>d.na-lac.</i> , nasolacrimal duct
<i>c.art.</i> , cartilago articularis of pterygoid	<i>epipt.</i> , epipterygoid
<i>c.ect.</i> , cartilago ectochoanalis	<i>f.ap.</i> , foramen apicale
<i>c.hyp.</i> , cartilago hypochiasmatica	<i>f.end.</i> , foramen endolymphaticum
<i>c.meck.</i> , Meckel's cartilage	<i>f.ep.</i> , foramen epiphaniale
<i>c.par.</i> , cartilago paraseptalis	<i>f.jug.</i> , foramen jugulare
<i>c.sph-e.</i> , cartilago sphenoethmoidalis	<i>f.mag.</i> , foramen magnum
<i>can.s-c.a.</i> , anterior semicircular canal	<i>f.n.VI</i> , foramen for nerve VI
<i>can.s-c.l.</i> , lateral semicircular canal	<i>f.n.VII</i> , foramen for nerve VII
<i>can.s-c.p.</i> , posterior semicircular canal	<i>f.n.VIII.a.</i> , foramen for anterior ramus of nerve VIII
<i>caps.jac.</i> , capsule of Jacobson's organ	<i>f.n.VIII.p.</i> , foramen for posterior ramus of nerve VIII
<i>cav.coch.</i> , cavum cochleare	<i>f.n.XII.a.</i> , anterior foramen for nerve XII
<i>cav.vest.a.</i> , cavum vestibulare anterius	<i>f.n.XII.p.</i> , posterior foramen for nerve XII
<i>cav.vest.p.</i> , cavum vestibulare posterius	<i>fen.b-c.p.</i> , fenestra basicranialis posterior
<i>ch.</i> , notochord	<i>fen.coch.</i> , fenestra cochleae
<i>co.</i> , concha	<i>fen.e-op.</i> , fenestra epioptica
<i>co.jac.</i> , concha of Jacobson's organ	<i>fen.hyp.</i> , fenestra hypophyseos
<i>col.</i> , columella auris	
<i>com.p-f.</i> , postfacial basicapsular commissure	

- fen.m-op.*, fenestra metoptica
fen.ol., fenestra olfactoria
fen.op., fenestra optica
fen.pr-ol.i., fenestra prootica inferior
fen.pr-ol.s., fenestra prootica superior
fen.s.na., fenestra superior nasi
fen.sep., fenestra in interorbital septum
fen.vest., fenestra vestibuli
fen.y., confluent fenestra epioptica, fenestra metoptica, and fenestra prootica
fis.m-ol., fissura metotica
fis.o-na., fissura orbitonasalis
foss.s-a., fossa subarcuata
ft.pl., footplate of columella
g.III, ganglion of nerve III
g.V.a., anterior ganglion of nerve V
g.V.p., posterior ganglion of nerve V
g.VII, ganglion of nerve VII
g.VIII.a., anterior ganglion of nerve VIII
g.VIII.p., posterior ganglion of nerve VIII
hy.ar., hyal arch
hy.cor., body of hyoid
hyp., hypophysis
in.i-c., incisura intercondyloidea
ins.pl., insertion plate of columella
ins.pl', dense connective tissue surrounding insertion plate of columella
n.I—n.XII, cranial nerves
n.I', small nerves from olfactory lobes
n.II.ch., optic chiasma
n.V.1, first ramus of nerve V
n.V.2., second ramus of nerve V
n.V.3., third ramus of nerve V
n.V.eth.e., external ethmoid branch of nerve V. 1
n.V.eth.i., internal ethmoid branch of nerve V. 1
n.VII.c.t., chorda tympani branch of nerve VII
n.VII.hy., hyomandibular ramus of nerve VII
n.VII.pal., palatine ramus of nerve VII
oc., occipital arch
os ang., angulare
os comp., complementare
os den., dantale
os fr., frontale
os max., maxillare
os na., nasale
os pal., palatinum
os par., parietale
os p-fr., postfrontale (postfrontale mediale)
os p-op., postoperculare (goniale)
os p-orb., postorbitale (postfrontale laterale)
os p-quad., paraquadratum (quadratojugale)
os pr-fr., praefrontale
os pr-max., praemaxillare
os pr-op., praeoperculare (operculare)
os pt., pterygoideum
os s-ang., supraangulare
os s-max., septomaxillare
os sq., squamosum
os tr., transversum
os vo., vomer
os zyg., zygomaticum
p.ext., pars extraconchalis of nasal capsule
p.i., pars inferior of insertion plate of columella
p.s., pars superior of insertion plate of columella
p.tr., pars triangularis of nasal paries
pi.ac., pila accessoria
pi.pr-ol., pila prootica
pl.ant., planum antorbitale
pr.ac.p., processus accessorius posterior of columella (processus interhyale)
pr.al.i., processus alaris inferior
pr.al.s., processus alaris superior
pr.asc., processus ascendens of tectum posterius
pr.b-pt., processus basipterygoideus
pr.int., processus internus of columella
pr.lin., processus lingualis of hyoid
pr.max.a., processus maxillaris anterior

PLATE 2

EXPLANATION OF FIGURES

2 Stage 5. Ventral aspect of chondrocranium, $\times 25+$. Membrane bones represented on right side.

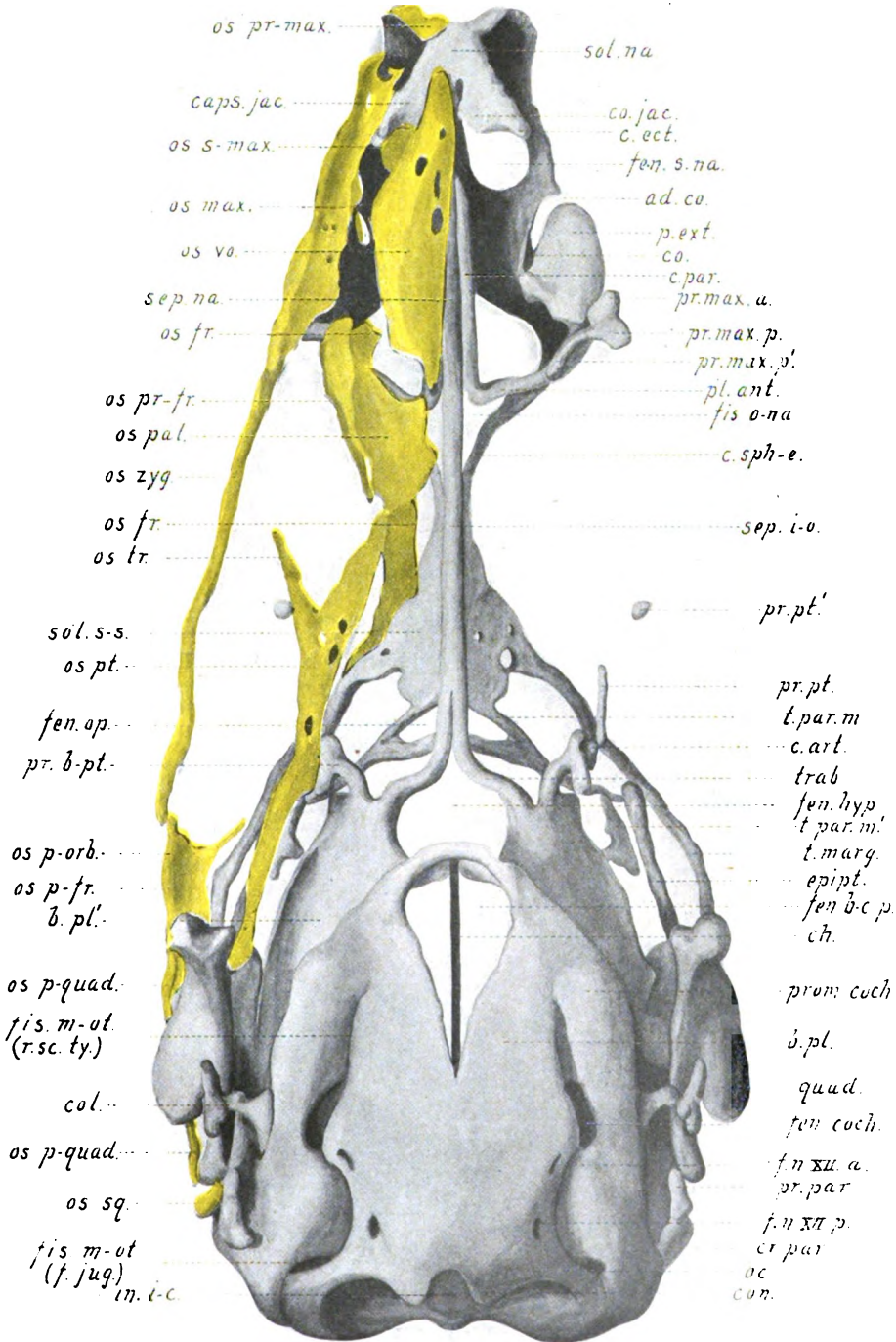


PLATE 3

EXPLANATION OF FIGURES

3 Stage 5. Left lateral aspect of chondrocranium, $\times 25+$. Bones omitted entirely and paired cartilages represented for left side only.

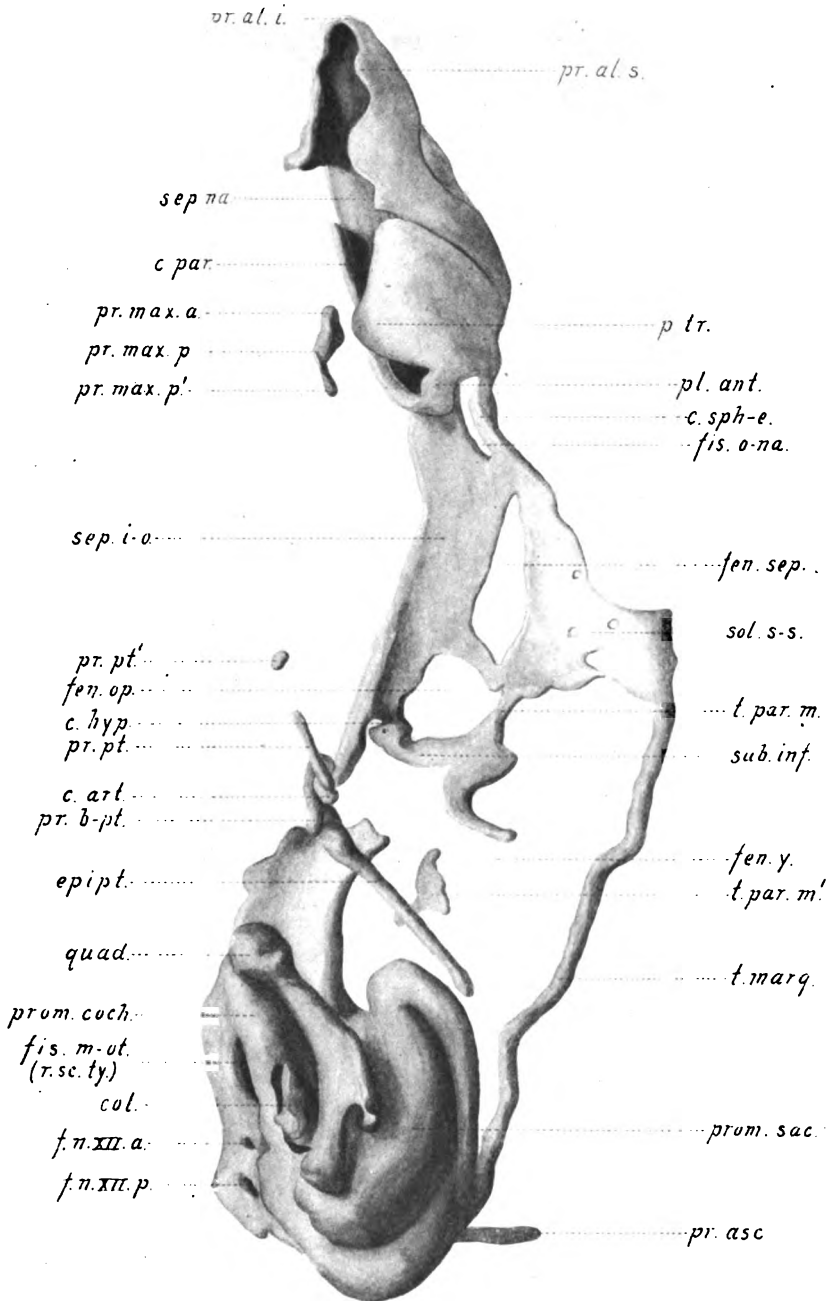


PLATE 4

EXPLANATION OF FIGURES

- 4 Stage 5. Dorsal aspect of lower jaw and hyobranchial apparatus, $\times 20$. Membrane bones represented on right side.
- 5 Stage 5. Lateral aspect of solid model ('cast') of cavity of otic capsule, $\times 40$. Note that fenestrae appear as projecting plugs.
- 6 Stage 5. Posterolateral aspect of occipital region, $\times 40$.

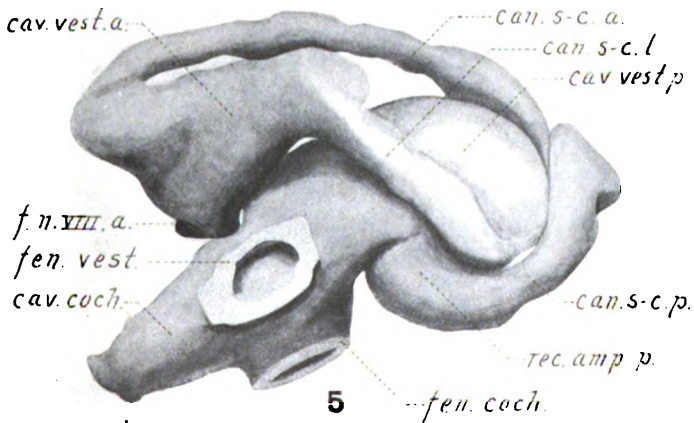
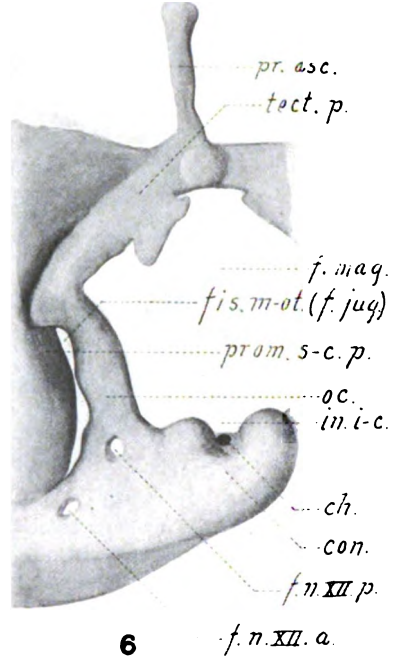
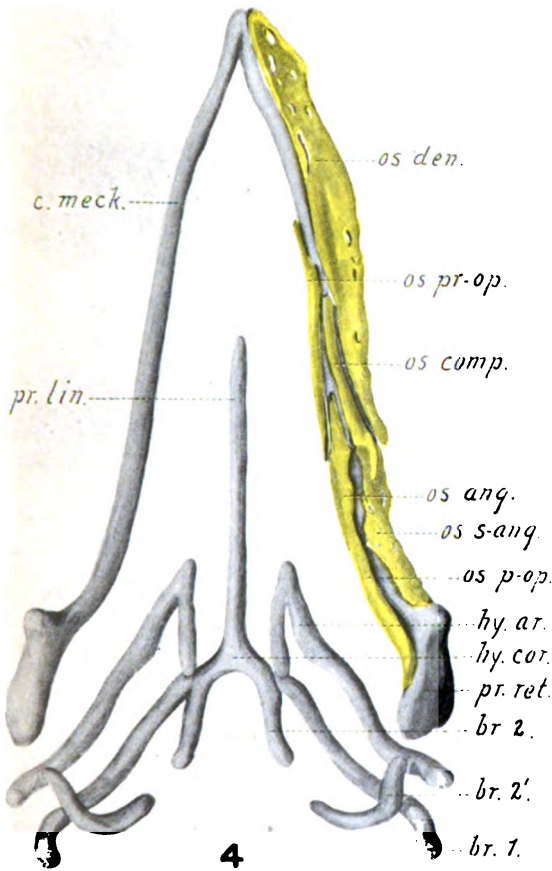


PLATE 5

EXPLANATION OF FIGURES

7 Stage 5. Ventrolateral aspect of otic capsule and columella auris, $\times 40$. Position of fenestra cochleae, invisible in figure, indicated by arrow. Drawing slightly oblique; dorsoventral orientation indicated by line *D-V*.

8 Stage 5. Dorsomedian aspect of otic capsule, $\times 40$. Position of fenestra cochleae, invisible in figure, indicated by arrow. Drawing slightly oblique: dorsoventral orientation indicated by line *D-V*.

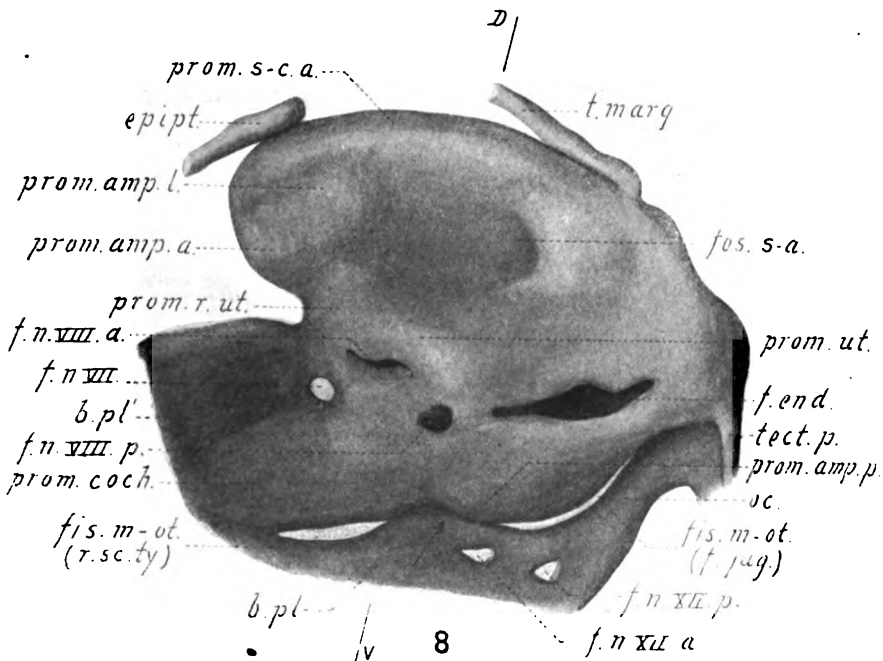
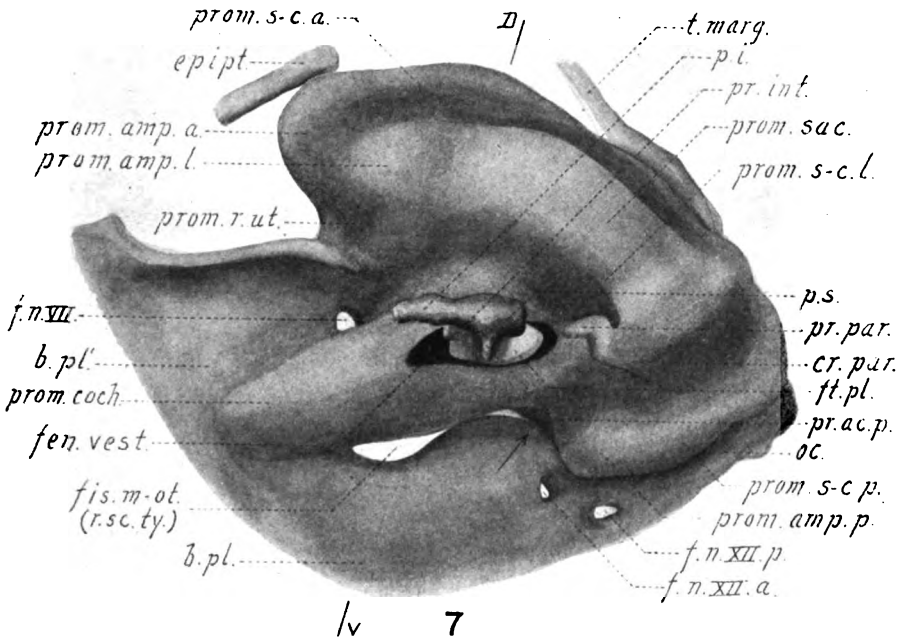
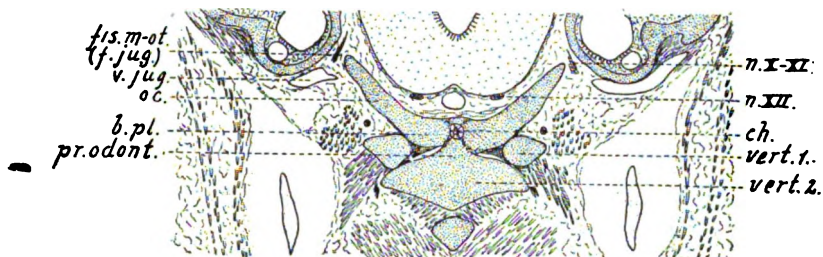


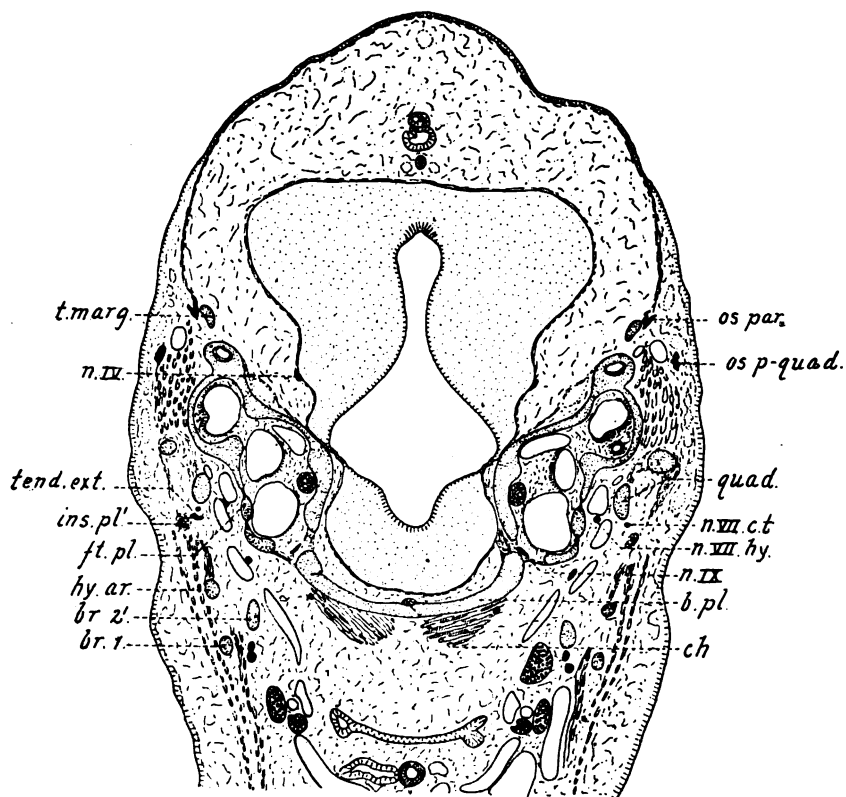
PLATE 6

EXPLANATION OF FIGURES

- 9 Stage 5. Transverse section (323) through craniovertebral articulation, $\times 30$.
- 10 Stage 5. Transverse section (297) through recessus scalae tympani, $\times 25$. Showing passage of nerve IX through recessus.



9



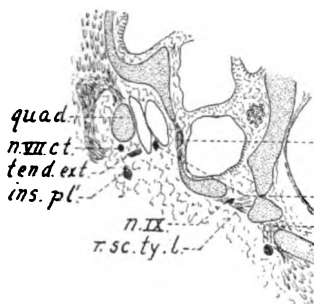
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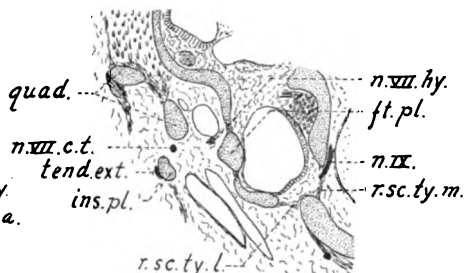
PLATE 7

EXPLANATION OF FIGURES

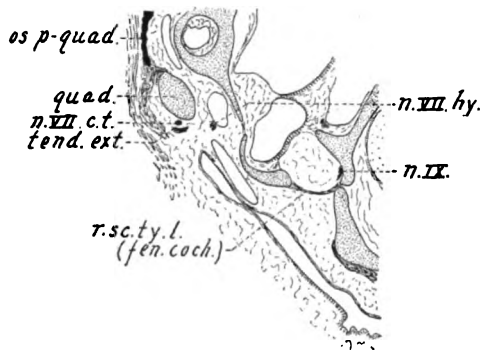
- 11 Stage 5. Transverse section (300) through recessus scalae tympani, × 30. Showing exit of nerve IX from recessus.
- 12 Stage 5. Transverse section (293) through recessus scalae tympani, × 30. Showing entrance of nerve IX into recessus.
- 13 Stage 6. Transverse section (410) through recessus scalae tympani, × 30. Showing intracapsular course of nerve IX. Compare with figure 10.
- 14 Stage 6. Transverse section (407) through recessus scalae tympani, × 30. Showing penetration of median wall of otic capsule by nerve IX. Compare with figure 12.
- 15 Stage 6. Transverse section (403) through recessus scalae tympani, × 30.



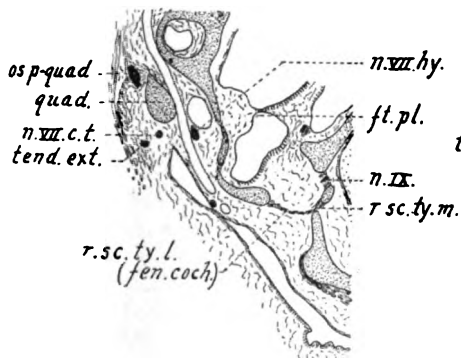
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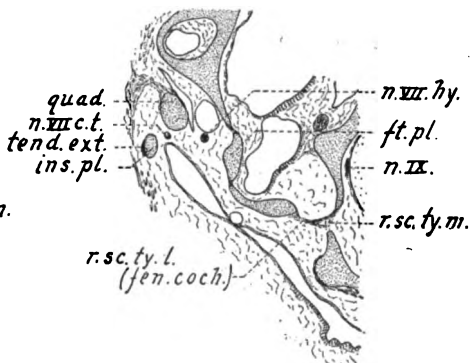
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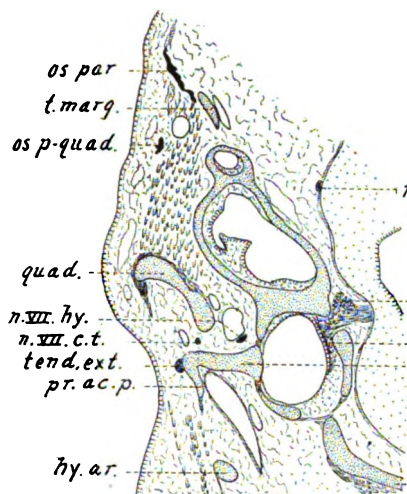


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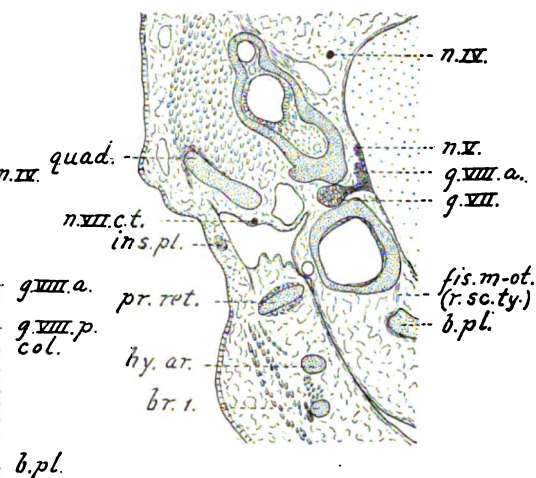
PLATE 8

EXPLANATION OF FIGURES

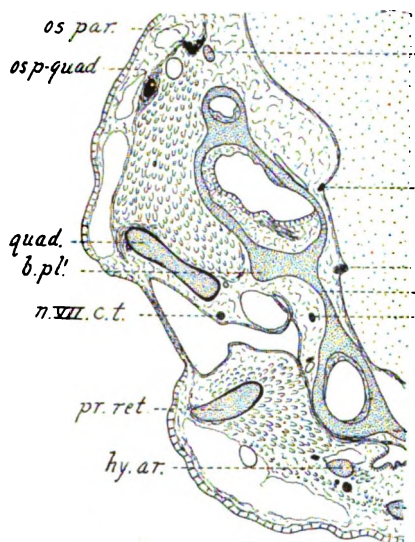
- 16 Stage 5. Transverse section (288) through columella auris, $\times 30$.
- 17 Stage 5. Transverse section (272) through facialis foramen, $\times 30$.
- 18 Stage 6. Transverse section (363) through prefacial basicapsular commissure, $\times 30$. Note intercapsular position of commissure.
- 19 Stage 5. Transverse section (245) through incisura prootica and trigeminus ganglia, $\times 30$.



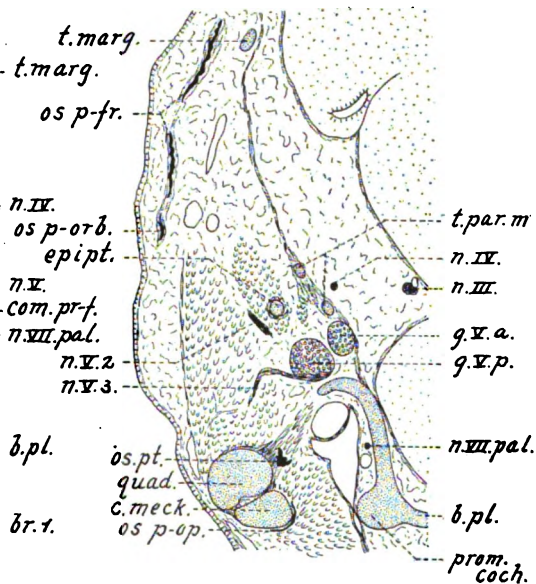
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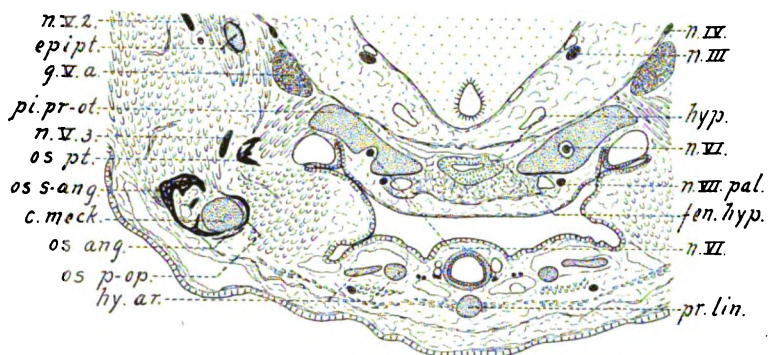
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PLATE 9

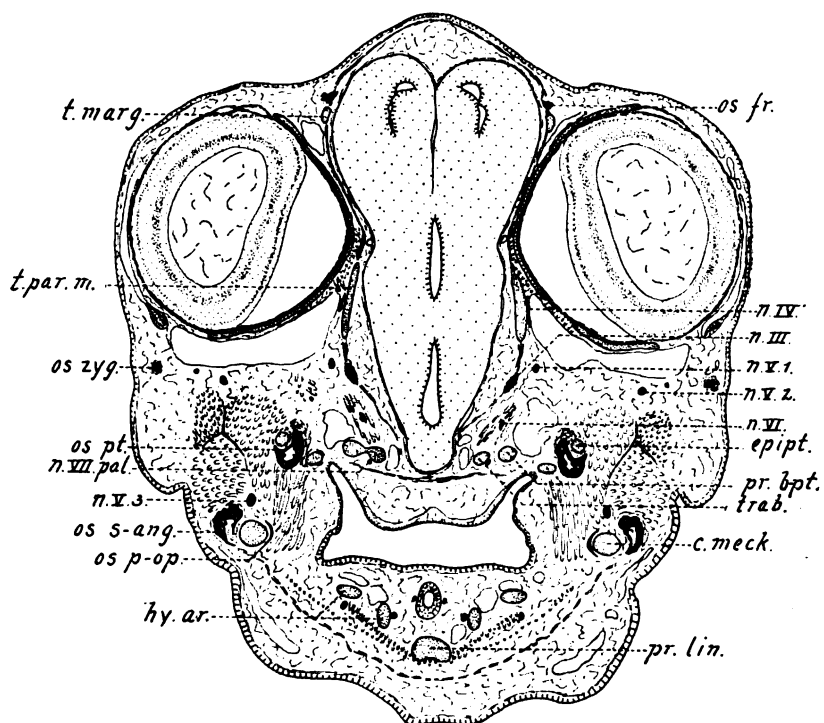
EXPLANATION OF FIGURES

20 Stage 6. Transverse section (309) through posterior edge of fenestra hypophyseos, $\times 30$. Note unusual relation of nerve VI to basal plate on left side of figure.

21 Stage 5. Transverse section (215) through roots of basipterygoid processes and trabeculae and through foot of epipterygoid, $\times 25$.



20



21

PLATE 10

EXPLANATION OF FIGURES

22 Stage 5. Transverse section (205) through subiculum infundibuli and articular cartilage of pterygoid, $\times 30$.

23 Stage 5. Transverse section (199) through cartilago hypochiasmatica and optic chiasma, $\times 30$.

24 Stage 5. Transverse section (168) through solum suprasedale and fenestra septi, $\times 25$.

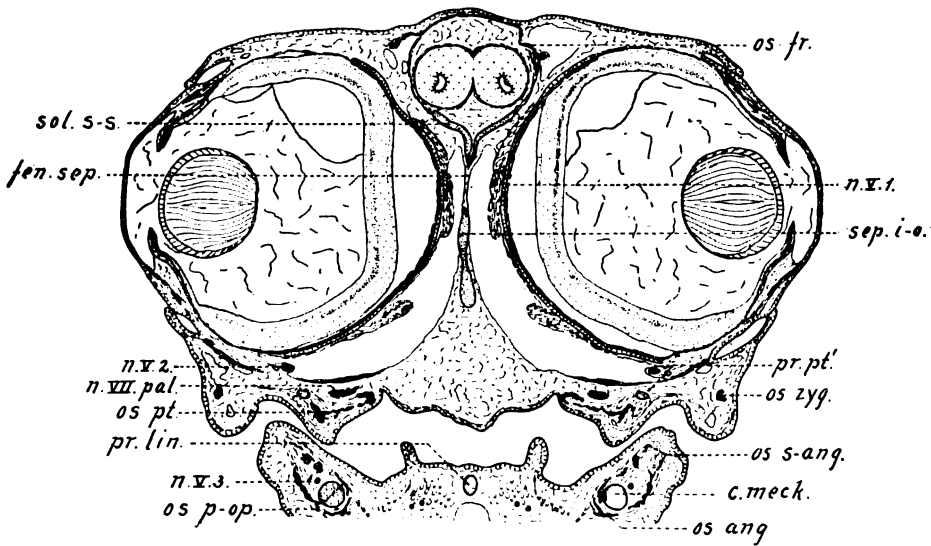
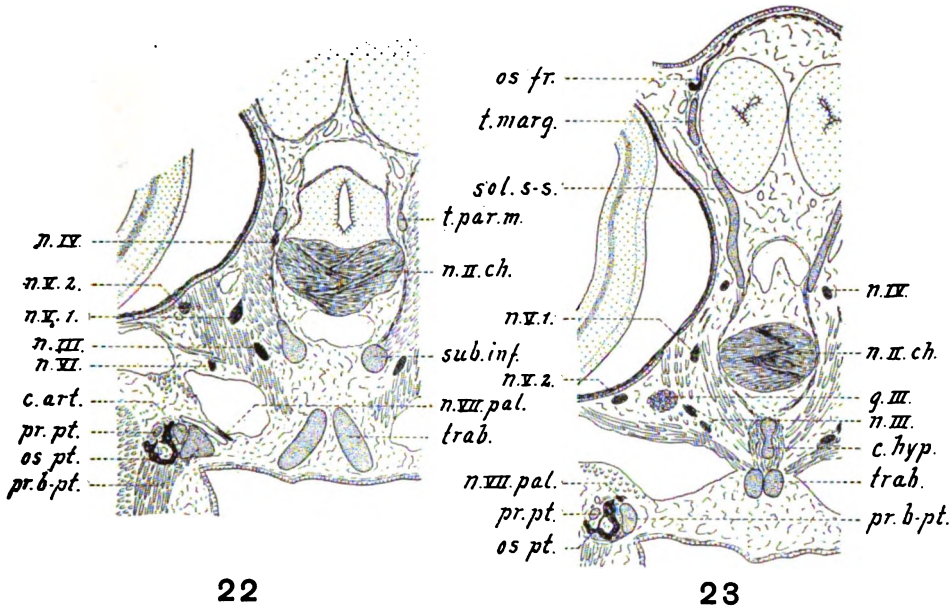
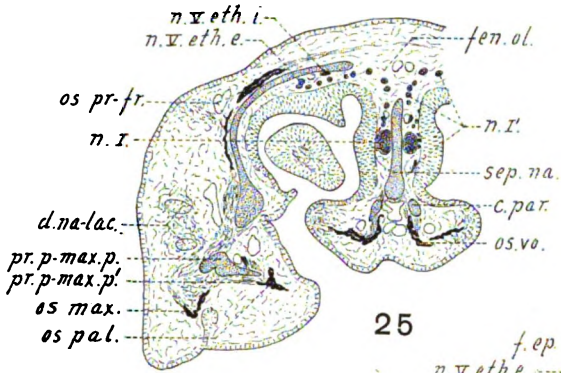


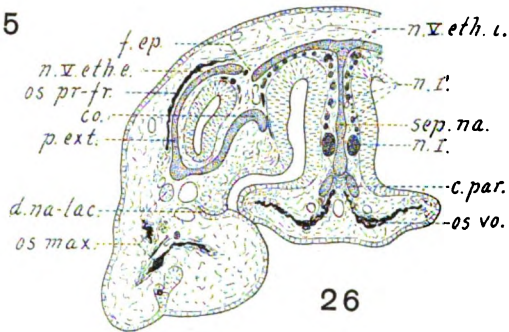
PLATE 11

EXPLANATION OF FIGURES

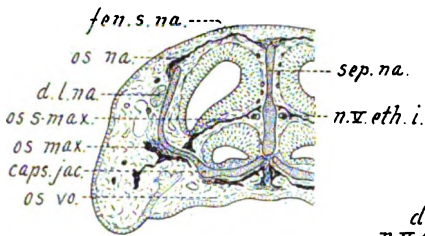
- 25 Stage 5. Transverse section (92) through processus maxillaris posterior,
× 30. Note crossing of process from os maxillare to os palatinum
- 26 Stage 5. Transverse section (80) through concha and foramen epiphaniale,
× 30.
- 27 Stage 6. Transverse section (46) through incomplete zona annularis,
× 30.
- 28 Stage 5. Transverse section (37) through concha of Jacobson's organ and
fenestra superior nasi, × 30.



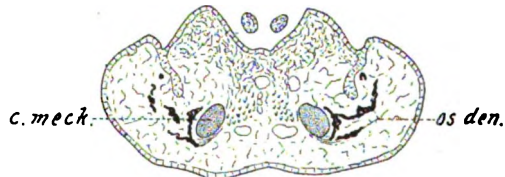
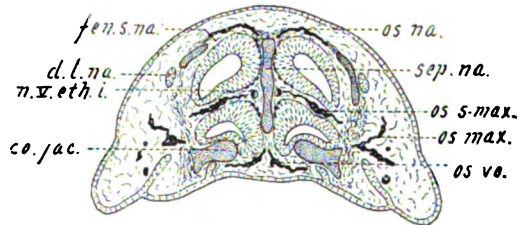
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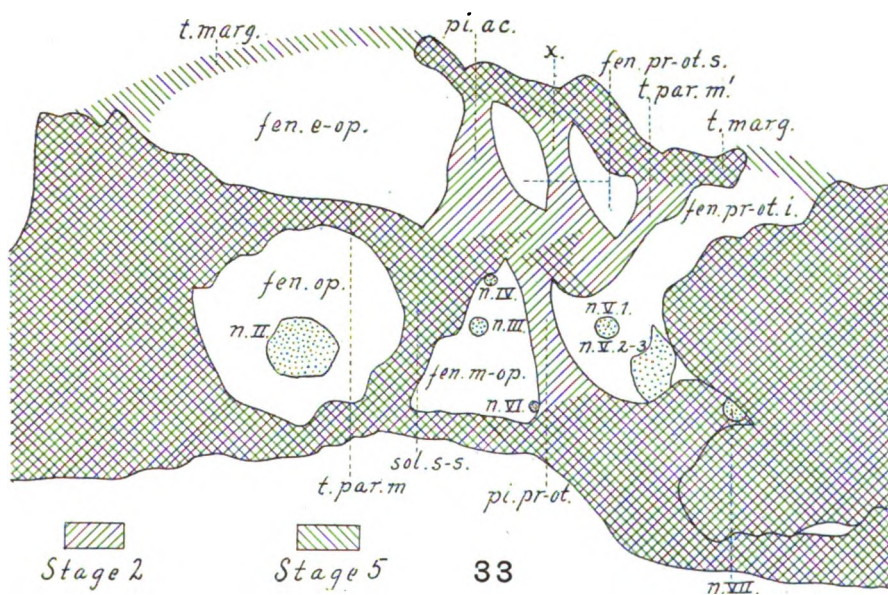
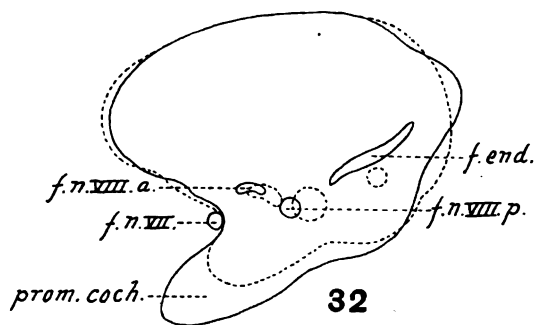
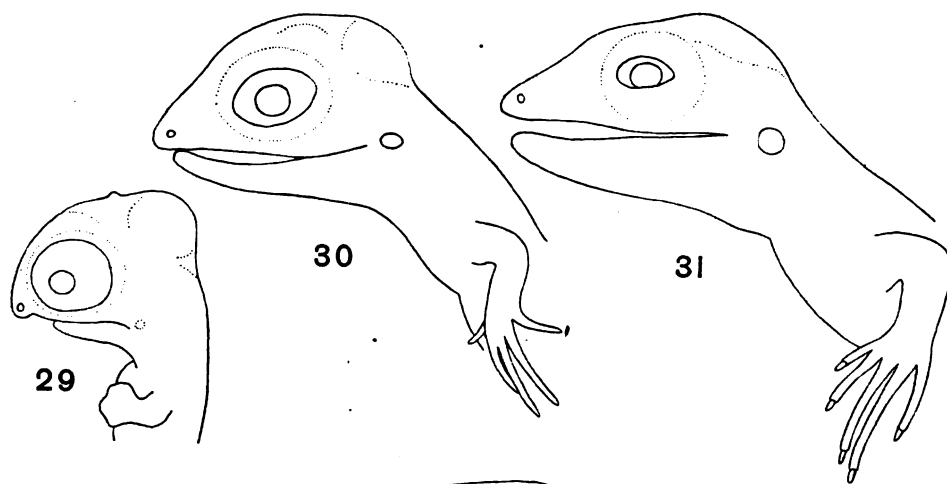
PLATE 12

EXPLANATION OF FIGURES

29 to 31. Stages 1, 5, and 6. Heads of embryos, $\times 7\frac{1}{2}$. Showing changes of size and form in stages studied.

32 Stage 5. Diagrammatic median view of ear capsule compared with that of *Lacerta*. The outlines for *Eumeces* (solid lines) are taken from figure 8 of this paper; those for *Lacerta* (dotted lines) from Gaupp's figure 7.

33 Stages 2 and 5. Diagrammatic view of lateral wall of temporal region of stage 5 superimposed upon that of stage 2. The outlines for stage 2 are based upon an accurate graphic reconstruction; the proportions of stage 5 are somewhat distorted to bring corresponding portions into coincidence. For fuller description, see page 175 of text.



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Resumen por el autor, William Harold Leigh-Sharpe.
Londres.

La morfología comparada de los caracteres sexuales secundarios de los peces elasmobranquios—los apéndices copuladores, sus sifones y glándulas. Memoria I.

El saco muscular llamado sifón en *Scyllium* etc., es homólogo del saco que contiene la glándula del apéndice copulador de *Raia*. El autor describe experimentos que demuestran que los sifones están normalmente llenos de agua de mar y están rodeados de músculos potentes cuya contracción inyecta en la hembra a los espermatozoides contenidos en los surcos del apéndice copulador, los cuales sufren un movimiento de rotación bajo la acción de una estructura en forma de abanico. El propósito de esta memoria y las que se publiquen ulteriormente es comparar bajo este punto de vista todos los elasmobranquios que puedan obtenerse. En *Scyllium catulus* existen parasifones. La histología de la pared del sifón, una descripción de los espermatozoides y algunos detalles nuevos de la glándula del apéndice copulador de *Raia* son objeto de descripciones en el presente trabajo, así como las observaciones sobre las particularidades del músculo estriado que se inserta en la glándula del apéndice copulador.

Translation by José F. Nonides
Cornell Medical College, New York

THE COMPARATIVE MORPHOLOGY OF THE SECONDARY SEXUAL CHARACTERS OF ELASMOBRANCH FISHES

THE CLASPERS, CLASPER SIPHONS, AND CLASPER GLANDS

MEMOIR I

W. HAROLD LEIGH-SHARPE, M. Sc. (LOND.)

TWELVE TEXT FIGURES¹

The present memoir deals with the following species:

<i>Scyllium catulus</i>	247
<i>Scyllium canicula</i>	252
<i>Acanthias vulgaris</i>	259
<i>Raia circularis</i>	260

In the male elasmobranchs, where fertilization is internal, the basal element of each pelvic fin (basipterygium) is prolonged to form a stout backwardly directed skeletal rod supporting a portion of the fin which is demarcated from the remainder and specially modified to form a copulatory organ, the clasper.

The clasper is rolled up in a manner resembling a scroll, so that instead of being a groove, as it is usually described, it is a sufficiently closed tube along the greater portion of its middle length, though the edges may not be and usually are not completely fused, but overlapping. This tube is one along which spermatozoa pass, injected by an apparatus, the siphon, which has not hitherto been sufficiently well known and investigated.

The anterior proximal opening into this scroll-like clasper groove or tube will be hereafter known for the sake of brevity as the apopyle, the posterior, distal exit from the same as the hypopyle. In the sharks and dogfish the apopyle is close to

¹ The figures were executed by Miss E. C. Humphreys from the author's dissections and preparations, except figure 4 by Michael G. L. Perkins, to both of whom best thanks are tendered.

the cloacal aperture, while in the skates it is some considerable distance posterior to it, an inch or more in a moderately sized adult.

Leading into the apophyle by a narrow aperture, so as to communicate with the clasper tube, on either side, is a large cavity, the siphon, a sac with extremely muscular walls, situated immediately below the corium of the ventral surface of the abdomen, frequently several inches in length, close to the median line, and ending blindly, having no communication with the coelom, and whose function and significance it will be my endeavor to elucidate.

In the skates, on the other hand, no such hollow sac is found, but its place is taken by the clasper gland, contained in a sac which it completely fills. This gland has long been recognized, but its containing sac does not appear up to the present to have been demonstrated to be homologous with the clasper siphon of the sharks and dogfish, which is but little known.

Other accessory structures may be present on the claspers, such as the spurs and the like in *Acanthias*, but of these none attains such importance and is more frequently present than a fan-like expansion at the distal end of the clasper, the rhipidion, whose function is to spray the spermatozoa in all directions in a radiating manner. If a jet of water, as from a garden hose, be directed against a flat surface, e.g., a piece of board, at such an acute angle as to be almost parallel with it, the water will fly off from the edges in directions corresponding to the shape of the margin of the board. If the board be fan-like at the edge, the water will radiate equally all round the quadrant. The rhipidion attains a greater development in the skates than in the sharks.

After this brief introduction, an insight into the topography and actual function of the siphons can best be gained by consideration of the first example under survey, *Scyllium catulus*, which will be treated more fully than the rest as a typical example.

It must be borne in mind, however, that the figures are not drawn so as to show the claspers in their natural position, but deflected in such a way (usually inward, though occasionally

outward) as to display the principal and accessory structures to best advantage.

SCYLLIUM CATULUS

The large spotted dogfish or nursehound

Figure 1 shows the relative positions of the structures enumerated in the introduction. The apopyle is so close to the cloaca

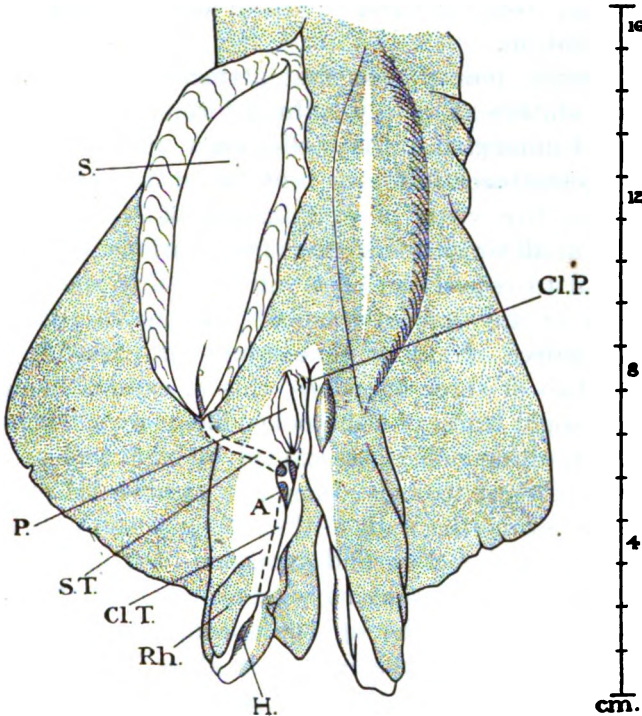


Fig. 1 *Scyllium catulus*. A., apopyle; H., hypopyle; S., siphon; P., parasiphon; S.T., siphon tube; Cl.T., clasper tube; Cl.P., cloacal pouch; Rh., rhipidion. (On the observer's right the siphon is shown actually inflated, the parasiphon diagrammatically so.)

that the spermatozoa which are constantly oozing from that aperture, at any rate in dogfish caught during the first half of the year, cannot fail to enter it of their own propulsion; beside which they are also sucked into it by a down-draught from the siphon, as will presently be demonstrated by experiment.

The large specimen upon which the following experiments were tried a day or so after it was caught was obtained at Plymouth in June, 1918, being only the second brought to the laboratory there that year. In this the siphon was 3 inches long.

The first explanation that would probably occur to an observer as to the use of the siphons is that the spermatozoa were either contained and stored in them or drawn into them previous to or during copulation.

My attention, indeed, was first attracted to the subject by the verbal statement of my valued former tutor and friend, Mr. J. T. Cunningham, the well-known authority on fishes, that "no spermatozoa had ever been found in these sacs at any time." After five years of examination of some hundreds of *S. canicula*, at all times and seasons from various parts of British coasts, I fail to record the observation of any spermatozoa in the siphons of any animal studied, whether of this or other species, or, indeed, of any contents whatever, parasites included, except, on two or three occasions, this *S. catulus* being one of them, very slight traces of what looked like mucus, but may have been decomposition debris, or exuded by the wall of the sacs themselves. I most strongly, therefore, endorse his proposition.

At the same time the walls of the siphon are so very muscular as to suggest the injection and expulsion of fluid of some sort, so I was reduced to considering whether or not that fluid might be merely sea-water. The siphons appear so early in development, in fact as early as the claspers, and are present in a young fish immediately after hatching, as also are the claspers in the male (fig. 3), that this seems evidence that they are correlated with them.

Accordingly I performed the following experiment: The finely drawn-out end of a ball-pipette, similar to those used for filling fountain-pens, was introduced by way of the apophyle, which, in the natural position of the claspers, is open, into the aperture of the siphon sac which is extremely narrow. The pipette previously filled with an ordinary injection fluid of powdered carmine in suspension (containing a small quantity of

dilute formalin), was discharged by squeezing the ball and the carmine injected into the siphon sac.

The undissected side of the specimen now became most distinctly dilated on the abdomen to a surprising degree, causing a bulging of the skin in that locality, proving that the injection fluid was actually entering the siphon, as indicated on the (observer's) right-hand side (fig. 1).

The pipette was now quickly withdrawn and the clasper bent smartly forward. In this position the apophyle is closed. Since the copulation of elasmobranchs takes place 'head to head' (a point for consideration in a later memoir dealing with the female), it is obvious that in nature the claspers of the male are always thus bent forward in copula. I imitated the natural angle as much as possible.

I next pressed lightly upon the distended siphon with a finger, simulating in this the natural muscular contraction of the living animal (an assistant having loosely ligatured the organ proximal to the apophyle). The carmine injecting fluid forthwith spurted out voluminously from the hypophyle to a distance of 3 or 4 feet, while none oozed out at the apophyle, though obviously not hampered by the presence of the ligature. Indeed, the presence of the ligature is not necessary to the success of the experiment. On repeating the experiment more slowly, it was easily demonstrated that a spiral rotatory motion was given to the ejected fluid by the presence of the rhipidion which is not very well developed in this species, though more so than in *S. canicula*; conversely, if the rhipidion be removed by dissection the fluid is not so rotated.

In order to simulate natural conditions as much as possible in the next experiment, the point of the pipette was inserted through the wall of the anterior end of the siphon sac and ligatured so as to preclude any possibility of leakage, and the whole fish immersed in a tank of water. It must be remembered that in life the siphon does not require to be filled as in the preceding experiment, since it normally contains sea-water.

To make conditions equal, precautions must be observed that escaping spermatozoa from the cloaca are not now drawn into

the siphon. This is easily done by closing the cloacal aperture by pressure of the finger. The siphon contents-water should now be tested and the absence of spermatozoa demonstrated. After death a slight pressure on the urogenital sinus is generally necessary to cause a flow of spermatozoa. If, now, the ball of the pipette be pinched and the stream of water ejected from the hypopyle be collected in a glass test-tube just sufficiently large to fit well over the tip of the clasper, the microscope will reveal the presence of spermatozoa in large numbers, accompanied usually by some of the eggs of the nematode that habitually infests the alimentary canal of the dogfish. This, I submit, is what occurs in nature, the ball of the pipette merely playing the part of muscular compression.

The clasper groove or tube is first filled with spermatozoa by a gradual flow through the apopyle. Copulation ensues, and the claspers are bent forward so that the apopyle is closed. Muscular contraction of the siphon wall follows, and the spermatozoa are ejected by the flush of sea-water into the oviduct of the female.

The posterior end of the oviduct is naturally dilated to admit of the inclusion of the claspers. In young females it is closed by a hymen. The rhipidion passes anterior to this dilation, and thus forms an organ of closer approximation, subserving another function.

The rhipidion is roughened on its outer border by a group of dermal denticles such as are found all over the skin, but pointing in the reverse direction. These doubtless serve to prevent elision of the claspers from the oviducts of the female and hence function as attaching organs. They are represented by stippling in the figure.

It will be as well to mention in this place that in all the figures the stippling indicates the presence of dermal denticles; the 'water-mark' or 'moirée' shading, muscles exposed in dissection; the unshaded portions smooth surfaces, such as the walls of the siphon cavity or, especially in the skate, skin devoid of denticles.

Though the correlation of the previously mentioned structures is sufficiently remarkable, matters are rendered still more complicated by the presence, at least in *Scyllium catulus*, of another pair of blind sacs, smaller in size, but of a similar nature to the siphons; situated in a like position, but nearer to the median line than the siphons proper, which, as far as I can discover, have never previously been described. They are vestigial in *S. canicula*, and only occasionally found in that animal. In order to distinguish them from the larger siphons, they will be referred to as the parasiphons (fig. 1, *P*).

While the siphonal tube is set at an angle to the apopyle, the parasiphonal tube is in the same straight line with that orifice. The apertures of the siphon tube and the parasiphon tube are situated in a common exedra.

I can but regard the parasiphons as accessory to the siphons. In the present case they are 0.9 inch in length, have similar muscular walls, and are capable of inflation and distention by a ball pipette, proving that they have no internal opening into the coelom. They are located dorsal to the skeletal rod of the clasper, and therefore in figure 1 their indicated inflation is purely diagrammatic.

Situated within the extraclacal aperture, on either side of the urogenital papilla are a pair of pocket-like depressions, the cloacal pouches, attached in front of which are the cloacal papillae with their apices directed backward. These structures are so well known to everybody as to need but passing mention. They are not present in all elasmobranchs.

Each papilla is perforated by a peritoneal canal, which leads anteriorly from the coelom, and opens posteriorly by the abdominal pores into the cloacal pouches. The pores are wide in this species, but are open only in mature dogfish.

The histological details of the structures under consideration are dealt with under *S. canicula*, material of that species being more available, while, in order to enable investigators to determine the presence or absence of spermatozoa in the siphons in reenacting the foregoing experiments, a figure of a spermatozoon is introduced (fig. 4).

SCYLLIUM CANICULA

The small spotted dogfish or rowhound (i.e., rough-hound)

This species, smaller than *S. catulus*, does not differ from the latter, except in the matter of trifling detail (fig. 2). The rhipidion is so reduced as to be practically negligible; while

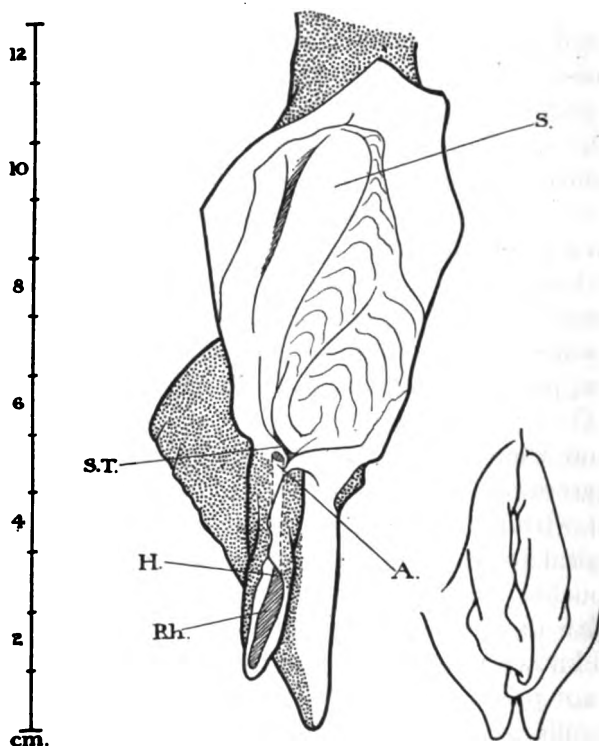


Fig. 2 *Scyllium canicula*. A., apopyle; H., hypopyle; S., siphon; S.T., siphon tube; Rh., rhipidion.

there is no doubt whatever that the siphon of the living animal contains sea-water, the presence of parasites adhering to the muscular walls within the cavity would go far to prove the matter. Howbeit, though I have examined some hundreds of *S. canicula* from all parts of the English Channel, from Plymouth, Bournemouth, Brighton, and elsewhere, I have not as

yet succeeded in obtaining any specimens of *Lernaeopoda scyllicola*, a copepod parasite, from within the siphon cavities, although that parasite is present almost without exception upon all the males, but not females, of *S. canicula* that are obtained, both in the extracloacal aperture (the usual place of attachment), behind the pelvic fins, and even on the claspers themselves, preferably upon the groove formed by their overlapping edges, and occasionally on the tips. Why their minute metanauplius larva is not occasionally sucked into the siphons is not yet clear, since it does not appear that the aperture leading into them is too small to admit of such a small organism. I think there is every possibility of such a discovery being made in the future.

As has already been mentioned, the siphons make their appearance very early in development. Figure 3 represents a transverse section through the abdominal region of a young *Scyllium canicula* the day after hatching. At this stage the claspers are already visible and may be distinguished with a lens; the section reveals the presence of the siphons.

In a young dogfish six days after hatching, the claspers are visible with the unaided eye and in length are about a quarter that of the pelvic fins.

For the benefit of those who may desire to repeat the experiments upon the siphons, figure 4 represents a spermatozoon of the dogfish, with that of the skate introduced for comparison. It consists of a head or nucleus (*n*), an apical body which is cytoplasmic (*a*), a cytoplasmic middle piece (*m*), around which is wound a spiral of chromatin, granular or rhabditiform in appearance, terminating in tail or flagellum (*f*), which is ribbon-like save for a filamentous end-piece (*e*). The tail (*e.f.*) is from three to three and a half times as long as the anterior portion (*a.n.m.*). The head and apical body are best seen in a film preparation stained with haemalum and eosin; the spiral is thrown into relief in a similar preparation stained with night-blue and allowed to dry; the tail and end-piece are best discerned in a similar preparation stained with night-blue and mounted in Canada balsam.

The characteristic spiral can be well observed in an ordinary film preparation made as follows: Spread upon a slide a thin smear of the fluid exuding from the urogenital aperture. Al-

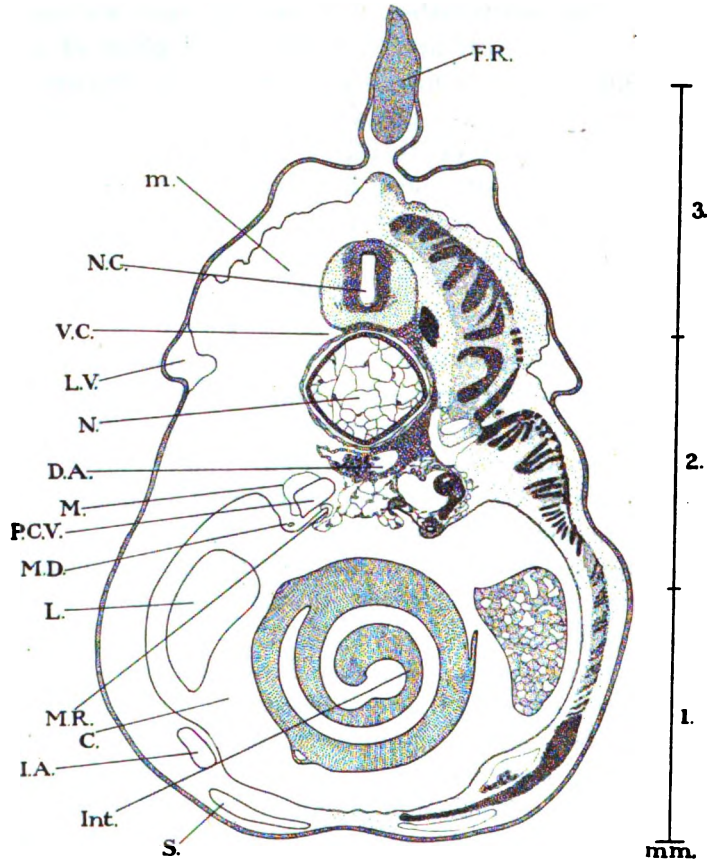


Fig. 3 *Scyllium canicula*, a transverse section through the abdominal region of an animal one day after hatching (borax-carmin). F.R., fin ray; m., myomeres; N.C., nerve cord; V.C., cartilage of the vertebra; L.V., lateral vein; N., notochord and its sheath; D.A., dorsal aorta; M., kidney (mesonephros); M.D., mesonephric (Wolffian) ducts; M.R., mesonephric (Wolffian) ridge; P.C.V., posterior cardinal vein; L., liver; C., coelom; Int., intestine; I.A., iliac artery; S., siphon.

low nearly to dry, but not quite, breathing on the surface to keep moist those peripheral parts which exhibit a tendency to desiccation.

Pour on the slide 90 per cent alcohol to fix. Allow nearly to dry, but not quite (as before). Stain: Mayer's haemalum, five minutes, sink in a basin of tap-water till purple. Dry all parts

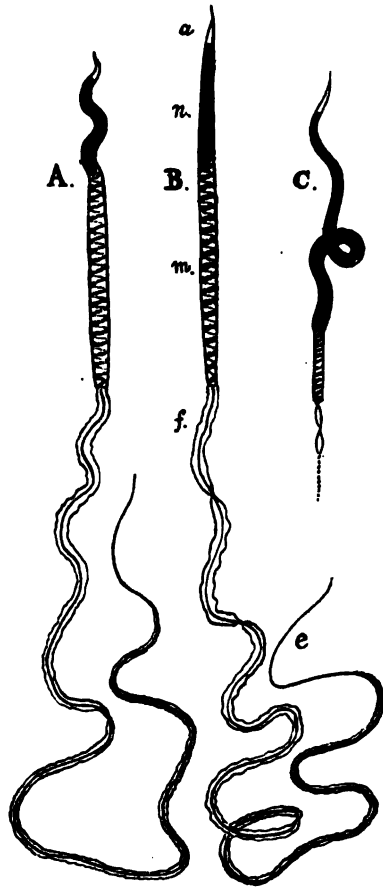


Fig. 4 Spermatozoa. A, B, of *Scyllium canicula*; C, spermatozoon of *Raja* (after Ballowitz). *a.*, apical body; *n.*, nucleus (head); *m.*, middle piece; *f.*, flagellum (tail); *e.*, end-piece; 1/12th inch objective. (Preparation and staining as explained in the text.)

of the slide except the film. Pour on successively: 30, 70, 90 per cent and absolute alcohol followed by xylol. Allow each liquid to remain on about a minute. Mount in Canada balsam.

The spiral can be distinguished in a temporary preparation without resort to staining.

The siphons arise in development (which will be dealt with in a succeeding memoir, since I am not yet sure as to the exact date of their definite appearance) apparently as invaginations of the ectoderm. The normal appearance of the body wall, for the purpose of comparison, is exhibited in figure 5. The epithelium is, of course, stratified. The tissues of the wall of the siphon

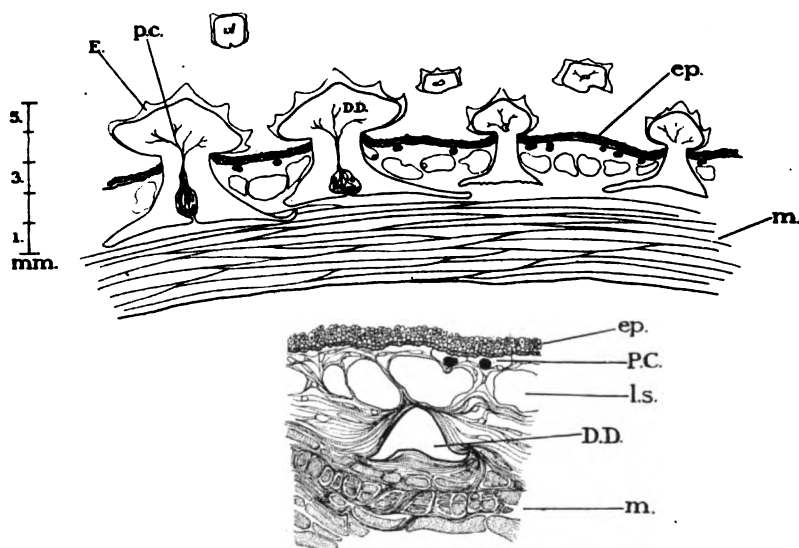


Fig. 5 *Scyllium canicula*. Longitudinal section through the body wall. *D.D.*, dermal denticles with *p. c.*, pulp cavity, and *e*, enamel; *Ep.*, epidermis; *P.C.*, pigment cells; *m.*, muscle; *l.s.*, lymph spaces.

are shown in figures 6 and 10. Here the epithelium is also stratified, and in the developing siphon (figs. 3 and 6) the band of muscle dorsal to the siphon should be noted. This is the muscle layer which exhibits such peculiarities mentioned later in the consideration of *Raia*.

The siphon wall consists thus of: internally, a membrane comprised of a stratified epithelium; outside this a broad layer of circular muscles, imbedded in which, on the ventral side, is a band of longitudinal muscles, while, dorsolateral in position, is

a longitudinal dorsal muscle-band external to the whole siphon and afterward to be described in *Raia* (fig. 11). These two longitudinal groups may have a coordinating function.

The parasiphons are vestigial in *S. canicula*. Out of one hundred specimens of this fish chosen at random indications of

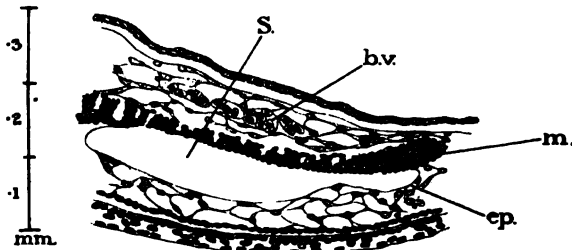


Figure 6

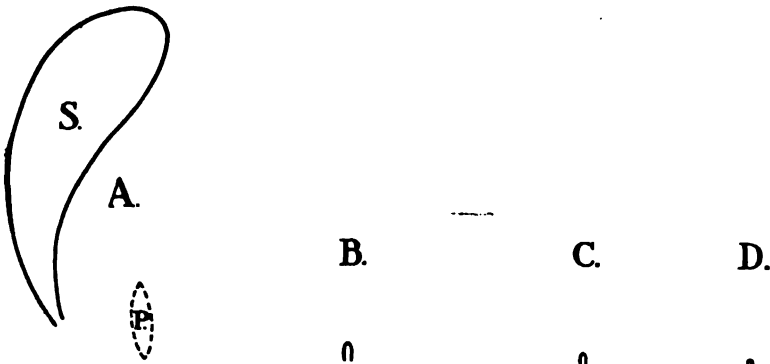


Figure 7

Fig. 6 *Scyllium canicula*, an enlarged view (from fig. 3) of the siphon in transverse section; *S.*, siphon; *m.*, muscle; *Ep.*, stratified epithelium; *b.v.*, blood-vessels.

Fig. 7 *Scyllium canicula*, a diagram of the relative proportions of vestigial parasiphons, as explained in the text.

parasiphons were recognizable in only thirty. In figure 7, diagram A represents the siphon (*S*) and a parasiphon (*P*) in dotted outline drawn to the same scale as it would appear if developed to the same extent as in *S. catulus*. Diagram B shows its vestigial development as exhibited by 3 per cent of the selected individuals; C as in 7 per cent; D as in 20 per cent; the remainder were without trace of parasiphons.

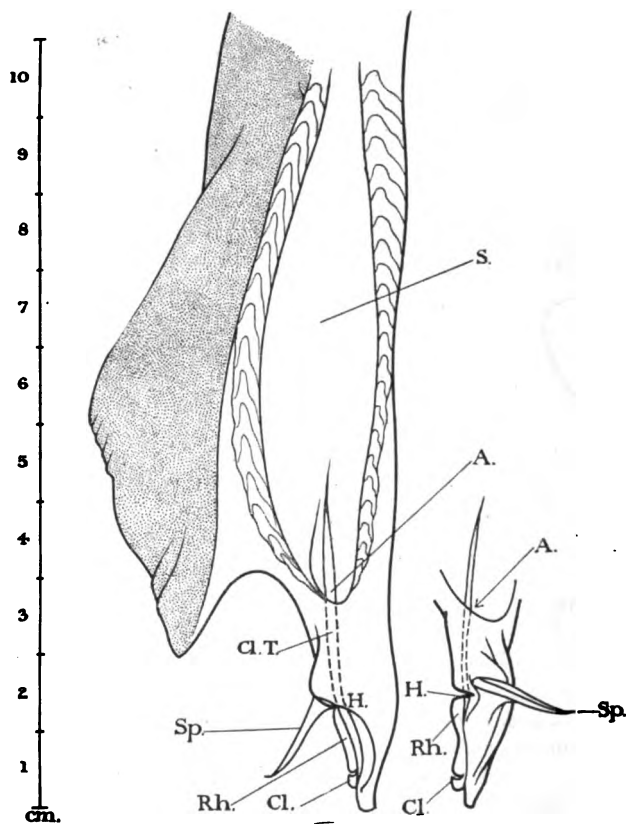


Fig. 8 *Acanthias vulgaris*. A., apopyle; H., hypopyle; S., siphon; Cl.T., clasper tube; Sp., spur; Rh., rhipidion; Cl., claw.

The posterior end of the skeleton of the clasper (myxapterygium) is provided with a basal joint which is capable of causing in the tip of the whole clasper a flexion inward, as is shown in the inset to the right in figure 2. This no doubt forms a means of attachment during copulation and is found developed in *Mustelus* to a greater degree, as will subsequently be shown.

ACANTHIAS VULGARIS

The spurred or spiny dogfish, or piked or picked dog

This siphon, for so large an animal, is small as compared with that of *Scyllium*, and situated more immediately beneath the skin in a space filled with loosely packed connective tissue, otherwise it presents no differences. In the accessory clasper structures this species exhibits the most striking divergences from *Scyllium*, as shown in figure 8, in which the relations between the various structures are indicated in two positions.

Six specimens taken at Plymouth in May, 1918, were examined the day or so after they were caught. No copepod parasites of any kind were observed upon them.

The apophyle is considerably removed from the cloaca. The actually closed portion of the clasper tube is short. The rhipidion is pronounced, but this type is chiefly remarkable for the presence of a short straight thorn-like spur, a brief distance anterior to the hypophyle. The spur is worked by a powerful muscle, so that while it normally is kept in a relaxed condition against the side of the clasper, once the latter has entered the oviduct of the female it can be erected, thus forming an important organ of attachment, penetrating, and even lacerating the tissues of its partner.

The extreme tip of the clasper, posterior to the hypophyle is also provided with a very small much-curved claw, which is likewise movable. It is difficult to see what attaching function this can possess. Possibly it may serve to rupture the hymen. This animal is provided internally with short globular sperm sacs instead of elongated thin ones as in *Scyllium*.

RAIA

TYPE: RAIA CIRCULARIS

The painted, ocellated or cuckoo-ray

In the skates the most striking divergences from the conditions that obtain in *Scyllium* are observed (fig. 9). The claspers and the ventral surface of the pelvic fins are almost devoid of

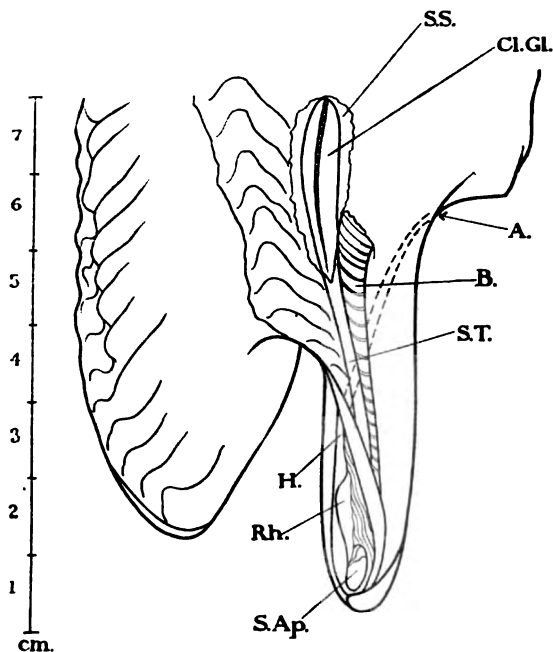


Fig. 9 *Raia circularis*. A., apopyle; H., hypopyle; S.S., siphon (sac); Cl.Gl., clasper gland; S.T., siphon tube; S.Ap., aperture of siphon tube; B., basipterygium.

dermal denticles. The apopyle exists rather in name for the purposes of comparison than in fact and is removed a considerable distance posterior to the cloaca. In the somewhat small specimen of *Raia circularis* taken at Plymouth in April, 1918, upon which these observations were made, this distance was as much as 1 inch.

The clasper tube, not being completely closed, is a groove rather than a tube. The skeletal support afforded by the prolongation of the basipterygium is very slight, which permits of the intromittent organ being the much more easily reflected.

The rhipidion is well developed and in the form of a fan. The siphon sac is similar, similarly situated and similarly developed to that of the Squalida, but its function is obviously different, since its cavity is occupied by a peculiar and characteristic gland, well known to most observers, which almost fills the sac. This gland is called the clasper gland, an appellation which I retain, but which does not seem to me to be very happily chosen. To the unaided eye the gland appears as a bilobed structure, having a longitudinal groove running along it giving it a superficial resemblance to a date stone. In the groove a single row of papillae can be detected. Each lobe of the gland is compound and can be resolved into component parts. It will presently be seen that the papillae are the apertures of these components. The siphon tube, which forms the ultimate duct of the clasper gland, does not debouch at the apophyle, but is continued as a completely closed passage the whole length of the clasper, down to its posterior extremity, where it opens by a separate aperture posterior to the hypophyle. It would seem, then, that the highly muscular walls of the siphon sac subserve the function of injecting the secretion of the clasper gland into the female, and not that of the propulsion of spermatozoa.

The clasper gland (figs. 10 and 11) consists of a number of components arranged alternately inter se on either side of the median groove in the same manner as the taste-buds of the circumvallate papillae of the mammalian tongue, so that each successive papilla, or exit from a component gland, belongs to a component of opposite sides. Thus in transverse section in figure 10 (which is inverted to agree with the dissection in fig. 9) the gland-component ducts on the (observer's) left lead up to the papilla, while the ducts on the right do not lead to the same papilla, but are ducts of another component. The component glands are compound, branching in one plane. The gland cells lining the lumen are roughly spherical and are almost completely

filled by large spherical nuclei which stain very deeply with methyl green and other dyes.

The posterior four or five components of the gland do not alternate with one another, but occupy the whole width of the

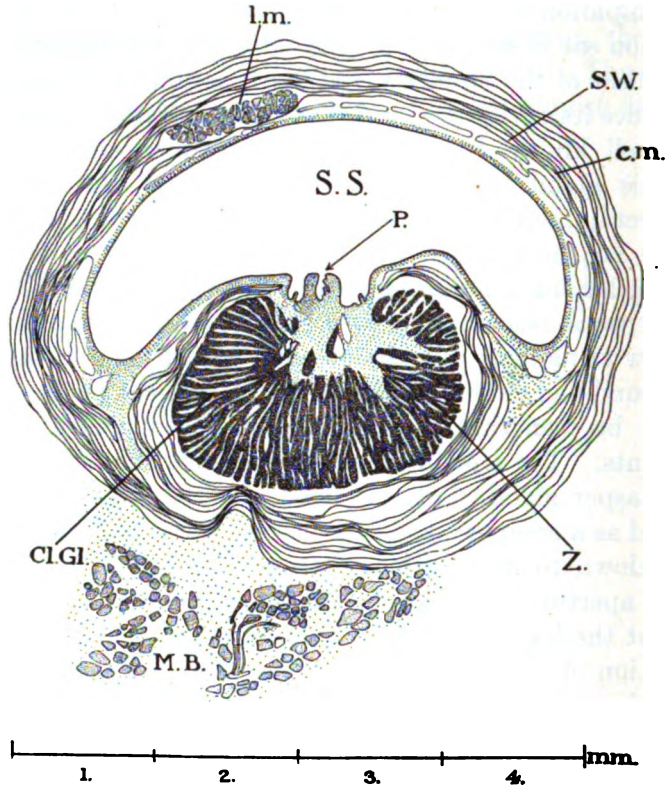


Fig. 10 *Raia circularis*, a transverse section through the siphon (methyl-green-eosin). *S.S.*, siphon sac; *S.W.*, siphon wall; *c.m.*, circular muscles; *l.m.*, longitudinal muscles; *Cl.Gl.*, clasper gland; *P.*, papilla of the component gland on the observer's left; *Z.*, another component of the gland; *M.B.*, dorsal muscle-bundle of the siphon.

organ which is considerably narrowed in this region. These components, moreover, are devoid of papillae, and all of their ducts lead obliquely forward so as to emerge at the most posterior papilla, that of the sixth component from the posterior end of the whole organ. This is shown in a horizontal section (fig. 11).

Attached to the siphon on its dorsal side is a large muscle band, which has already been alluded to in *Scyllium*. It is composed of fibers which are more markedly striped than any I have hitherto observed in the Vertebrata, and, in addition, possess two peculiarities: they exhibit 'nodes' where presumably one muscle cell adheres to its neighbor, the distance between the nodes decreasing in an appreciable manner as one approaches

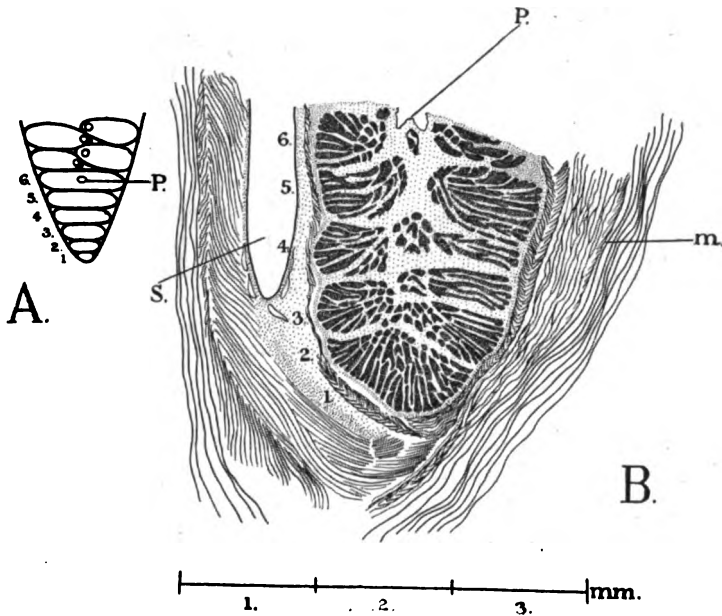


Fig. 11 *Raia circularis*. A., a diagram; B., a horizontal section through the six posterior components of the clasper gland (haemalum-eosin). 1, 2, 3, 4, 5, 6, the six posterior components of the gland numbered from the posterior end; P.; the papilla of the sixth component; m., muscle; S., siphon.

the point of insertion of the whole fiber on the siphon wall, so that the final internodes are small, and the terminal section ends bluntly in a digitate manner, subserving thereby functions analogous with a tendon (fig. 12). The point of origin of the siphon muscle is the pelvic girdle (pubic bar), and such a musculature suggests that it may be a retractor muscle for the protrusion and invagination of the siphon and its gland, although

the siphon tube is too narrow to admit of complete eversion. The muscle band is dorsolateral in position (figs. 6 and 10) on the outer side of the middle line.

Attention has already been drawn to the circumstance that the claspers of the skate possess a minimum of skeletal support. On the other hand, they are provided with an excess of erectile tissue (to be figured for another species in an ensuing memoir).

I have been present at Plymouth, at the trawling of a specimen of *Raia circularis*, taken immediately after copulation, in which the claspers were swollen to four times their natural size, and appeared of a rose-pink hue due to a suffusion of blood.

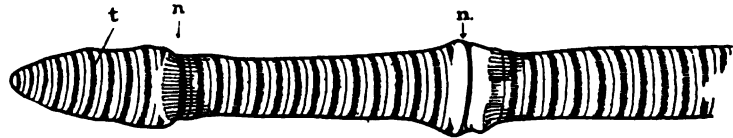


Fig. 12 *Raia circularis*, a single strand of striped muscle cells from the dorsal muscle bundle of the siphon (Farrant's medium, unstained). *n.*, nodes; *t.*, terminal cell functioning as a tendon.

Such an erection is not needed in *Scyllium* and *Acanthias*, which are provided with dermal denticles and spurs, respectively, while *Raia* relies on this phenomenon and on a relatively larger rhipidion for fixative purposes during impregnation.

Such are the elementary facts, several of them new, drawn from the four most common representatives of elasmobranchs found around British coasts. It is when we consider the problems arising out of these, and compare these types with other less known and intermediate forms, that the subject grows more interesting.

LITERATURE

The chief standard works on the claspers of the elasmobranchs are:

JUNGENSEN, H. F. E. 1898 a Ueber die Bauchflossenanhänge (Copulationsorgane) der Selachiermännchen. *Anat. Anz.*, Bd. 14.

1898 b Om appendices genitales hos havkalen, *Somniosus microcephalus* Bl. Schn., og andre Selachier. *Danske Ingolf-Eped.*, vol. 2, no. 2.

HUBER, O. 1901 Die Kopulationsglieder der Selachier. *Zeitsch. wiss. Zool.*, Bd. 70.

Jungersen deals almost solely with the musculature and the skeleton of the claspers, in some species with the skeleton exclusively. The siphon (referred to here as the 'glandular sac') is only mentioned in passing, except in the case of *Rhina squatina*, where it is figured partly dissected in situ. No histological details are given.

Huber's paper is on the same lines as Jungersen's, but the external features of some of the claspers are figured. The species, however, differ from those dealt with in the present paper.

BOLAU, C. C. H. 1881 Ueber die Paarung und Fortpflanzung der Scyllium Arten. *Zeitsch. wiss. Zool.*, Bd. 35.

This is the first paper giving an account of an observation of the copulation of an elasmobranch in an aquarium. It states that only one clasper was inserted at a time and that the coition lasted twenty minutes.

AGASSIZ, LOUIS 1858 (On the reproduction of Selachians.) *Proc. Boston Socy. Nat. Hist.*, vol. 6, p. 377.

Agassiz first formulated the idea, later widely copied, that the siphon is a reservoir for spermatozoa. This opinion was based upon no evidence.

SCHNEIDER, ANTON F. 1883 Ueber die Begattung der Knorpelfische. *Zool. Beiträge*, Bd. 1.

Schneider was the first to state that he had found spermatozoa (quantity not noted) in the siphons of *Acanthias* and *Callorhynchus*. No proof was given. He does not appear to have made a microscopical examination to determine what he saw was spermatozoa or was merely a mucous secretion of the walls of the siphon.

REDEKE, HEINRICH CARL 1898 Het urogenitalsystem der Selachier en Holocephalen. *Akad. Proefschrift*, Helder.

Redeke states that in *Mustelus*, postmortem, he found the siphon half full of spermatozoa. There is no suggestion as to how they entered. In my laboratory experiments on *Scyllium catulus*, described above, an explanation is given of how this may have happened.

Resumen por el autor, Franz Schrader.
Universidad Columbia, Nueva York.

Determinación del sexo en *Trialeurodes vaporariorum*.

Las recolecciones de individuos hechas al azar demuestran una distinta superioridad numérica de las hembras sobre los machos. Los experimentos de cría demuestran que las hembras no fecundadas originan machos, mientras que las que se han apareado producen ambos sexos en proporciones variables. El proceso de maduración es semejante en todos los óvulos, habiendo una división reductora y otra ecuacional. Si el óvulo no está fecundado se desarrolla con el número haploide de cromosomas y produce un macho; si está fecundado se restablece el número diploide produciéndose una hembra. En la espermatogénesis parece haberse eliminado por completo la división reductora. Las inclusiones celulares que aparecen en las células del micetoma de ambos sexos parecen ser de la misma naturaleza que otros hongos simbióticos ya descritos en varios insectos.

Translation by José F. Nonides
Cornell Medical College, New York

SEX DETERMINATION IN THE WHITE-FLY (TRIALEURODES VAPORARIORUM)

FRANZ SCHRADER

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FOUR PLATES (THIRTY-SIX FIGURES)

INTRODUCTION

Parthenogenesis in the Homopteran family of Aleyrodidae has been known for some years. In 1903, A. W. Morrill, in the course of some breeding experiments with *Trialeurodes vaporariorum*, found that all of the eggs laid by virgin females gave rise to males. Later, working with E. A. Back ('11), he discovered that the same held true for *Dialeurodes citri*, another member of the same family. Both of these cases were reported from America. In apparent contradiction to these conclusions, E. Hargreaves ('14), working on *Trialeurodes vaporariorum* in England, found that there only females emerge from eggs laid by virgin females.

Recently, C. B. Williams ('17) confirmed Hargreaves' observations in regard to the English form, but while on a trip to America also found the reports of the American investigators to be correct, basing both of these conclusions on sex ratios obtaining in random collections. In England, these collections were made in various greenhouses, and of some 500 specimens obtained, only 2½ per cent were males. The American collection was made in Virginia, and there 36½ per cent of 254 specimens were males. My own collections, made in the vicinity of New York City, showed that the males constituted 46½ per cent of a total of 403. These counts are a sufficient indication of the fact that the earlier observations in regard to parthenogenesis in the species are not erroneous and that the parthenogenetic behavior is different in the two countries.¹

¹ N. R. Stoll and A. F. Shull, *Genet.*, vol. 4, in more extensive breeding experiments confirm the production of males from virgin females and find that the ratio of sexes in offspring from mated females is variable.

The only doubt which may exist arises from the question whether the same species is concerned in both countries. In this connection, A. C. Baker, of the U. S. Bureau of Entomology, having gone over some of Williams' English specimens, assures me that they are morphologically indistinguishable from the species occurring in America. We are therefore concerned with two lines of a species, which differ in their parthenogenetic behavior.

That these lines or races are not strictly confined within definite geographical limits may be indicated by an exceptional occurrence at Merton, England, where Williams found that over 34 per cent of 287 specimens were males. He believes, however, that they may have been introduced with some recently acquired plants. Unfortunately, all the families raised from virgin females of these exceptional white flies died before their sex could be determined, and no subsequent breeding experiments were made. I have not found the converse case to this in my work here, but think it perfectly possible that an occasional strain of parthenogenetic female producers may exist.

Very few observations have been reported on the offspring of mated females. Williams succeeded in raising two very small broods from mated females which came from the exceptional Merton strain. In these broods, as in broods which I have raised from mated American females, both sexes were represented. Mr. Williams informs me that he never observed copulation in specimens of the ordinary English stock. The broods produced by females used in such experiments were always exclusively female, but, since it is questionable whether fertilization had taken place, no conclusions can be reached from such data.

Summed up, we thus have the following:

1. *Trialeurodes vaporariorum* has two races, one of which gives rise by parthenogenesis to males (America), the other to females (England).
2. Mated females of the male producing race give rise to both sexes.

The present work has been confined to the race occurring in America. I am indebted to Prof. E. B. Wilson and Dr. A. H. Sturtevant for aid and encouragement.

TECHNIQUE

Adults and all the younger developmental stages can be obtained without difficulty from a great variety of plants, especially from members of the group of Solanaceae. In order to obtain eggs for the study of maturation, I found it best to put a number of females into a vial containing a small leaf of one of their food plants. If previously starved for half an hour, they will readily settle on the leaf to feed and lay eggs, which after a little practice can then be picked off under a binocular by means of a sharp curved needle.

The eggs were fixed best with Gilson-Carnoy's fluid (equal parts of chloroform, absolute alcohol, and acetic acid, saturated with sublimate), their chitinous shell making other agents practically valueless. Their diminutive size makes puncturing almost impossible. The spermatogenesis, best studied in pupae of the third instar, offers considerable difficulty, arising from the smallness of the cells and the clumping of chromosomes. The fluids of Flemming and Hermann—used both singly and in conjunction with urea—and the fluids of Carazzi, Champy, Bouin, and Gilson-Carnoy, sublimate acetic, alcohol acetic—with various modifications of these in respect to dilution and temperature, were tried with little success. Gilson's fluid gave a few good preparations, while the Bouin and urea method of Allen was more consistent and successful than any of the others. The study of fresh material with aceto-carmin offered no advantages. I could find no differences in fixation due to the various methods of killing. In any case, it is absolutely necessary to make an incision close to the gonads, and preferably on both dorsal and ventral sides. Unless this is done, penetration of the fixing agent is always poor.

Sections of 6μ were found best for the study of the maturation phases in the eggs, while sections for the spermatogenesis were cut 3μ to 5μ thick. Hematoxylin without a counterstain proved to be the best stain.

MATURATION IN THE EGG

The earliest stages of maturation are found in the adult female, the abdomen of which is best fixed and sectioned entire. During the growth stages, the nucleus is situated at the center of the egg. Its black nucleolus, surrounded by an unstained protoplasmic area, and its definite nuclear wall make it readily distinguishable from the mass of densely staining yolk granules which fill the egg at this time. The prophase offers nothing that is new, the chromatin going through the various phases of chromosome formation while the nucleus approaches the central periphery of the egg. Here the nuclear wall disappears, and further developments take place in a clear irregular area of protoplasm, as in many other insect eggs.

Tetrads were found in a few of the preparations, their appearance resembling those of typical Homoptera as described by Miss Boring ('07). They are eleven in number, and their further condensation gives rise to the rounded deeply staining bodies which occur in equatorial plates of the first division. Although differing only very slightly in size, their arrangement in the plate seems to be a very definite one, and is the same in all eggs. Normally the metaphase is the farthest stage reached while the egg is in the body of the insect, but occasionally early anaphases are found in females which have been prevented from laying for a short period.

In the first division, each of the chromosomal bodies undergoes division and eleven dyads go to each pole. The outer group of these, or first polar nucleus, is not extruded, but remains close to the periphery. Both the polar nucleus and the egg nucleus enter immediately on a second division. The division of the former appears to lag somewhat behind, as the irregular arrangement of the dumb-bell-shaped dyads in figure 7 and in other preparations would indicate, but two daughter bodies are formed from it in any case. The division of the nucleus proceeds normally. The resulting second polar nucleus, like the first now divided, is not extruded, but remains close to the periphery, where all three ultimately disintegrate, while the reduced egg nucleus, with its eleven univalent chromosomes, travels toward the center of the egg.

PARTHENOGENESIS

Up to this point all eggs behave in exactly the same manner. The subsequent processes in the egg-nucleus depend on whether the egg has been fertilized or not. If no spermatozoon has entered the egg, a well-defined nuclear wall is formed around the matured egg-nucleus, and the chromosomes enter a semi-resting stage in which they become less sharp and definite in outline. They, nevertheless, still can be counted. Soon after, the rounded form is lost and the chromosomes lengthen, assuming the shape typical of those found in the dividing somatic cells of all the succeeding developmental stages. The first segmentation division proceeds normally, each of the first two nuclei receiving eleven chromosomes.

FERTILIZATION

If the egg has been fertilized, the egg-nucleus, as in the unfertilized egg, likewise approaches the center. The condition and position of the spermatozoon previous to this stage is very difficult to determine. Various sections showed what may possibly be the sperm head in a resting condition, but identification is never certain, because of possible confusion with the equally stained yolk material. Now, however, the sperm nucleus becomes apparent. At first still retaining the lengthened and pointed form of the sperm head, it gradually rounds out, undergoing the ordinary process of chromosome formation. When the two pronuclei meet, they are equal in size and each shows eleven lengthened chromosomes. The nuclear walls then break down and the twenty-two chromosomes are inclosed in an irregular vesicle. The first segmentation is regular in character, as before, and results in two nuclei, each with twenty-two chromosomes.

THE CHROMOSOMES SUBSEQUENT TO MATURATION AND
FERTILIZATION

Cytological investigation of such forms as *Ascaris* and the bee has demonstrated the possibility of formation of multiple chromosomes, that is, products of the union of two or more chromo-

somes, which are found during maturation and fertilization. These then appear as single chromosomes, but betray their compound character later when they break up into the component units again. In view of this fact, I traced out the behavior of the chromosomes in the present case with some care.

In the fertilized egg with its twenty-two chromosomes, the first segmentation division is followed by others which are perfectly regular. The first nuclei, still situated in the interior of the egg, all show twenty-two chromosomes. After the formation of about twelve such nuclei, these approach the periphery, and further divisions there establish the blastula. In these early stages, and up to the late blastula, division of all the cells occurs at practically the same time. In favorable preparations, therefore, the chromosomes in a great many cells of an embryo can be counted. One of my preparations shows such a condition, and the number can be made out definitely as twenty-two in some cases, and at least closely estimated to be such in many other cells. This constancy in the number of chromosomes in any single embryo is found also in the gastrula stage, where, however, divisions do not occur so rhythmically and the cells are smaller. Finally, pupae representing the various instars frequently show a number of cells in which the chromosomes are countable, and if twenty-two chromosomes are seen in one cell, every other cell in which the chromosome number can be made out will show the same number. Older pupae, that is, those in the fourth and latter part of the third instar, having the gonad in an advanced stage of development, always prove to be females in such cases, as an examination of the reproductive organs will show. I have never been able to make an absolutely certain count of the oogonial chromosomes, although very good estimates can be made in some cases. The number is here also twenty-two to all probability—certainly more than eleven.

The unfertilized egg, like that which has been fertilized, shows no irregularities following the maturation divisions and the smaller number of chromosomes makes their counting easier. As before, after the first few divisions, which here show eleven chromosomes, the nuclei travel to the periphery and the blastula

is formed. The latter, as well as the gastrula and the various pupal stages, show perfect constancy in the chromosome number. And in this case, finally, the older pupae, which show eleven chromosomes in the somatic cells, are found to be males with testes partly or fully developed. The only exception to the constancy in the number of chromosomes of the various somatic cells is furnished by the pseudovitelline cells or mycetoma, to which I will refer later.

Although I realize fully the danger of laying too much stress on chromosome counts of somatic cells, their remarkable constancy and definite coincidence with sex in this case cannot be altogether without significance.

SPERMATOGENESIS

The spermatogenesis presents considerable difficulty, due chiefly to the diminutive size of the cells and the tendency of the chromosomes to clump. In the hope of finding more favorable material, I also investigated a number of other genera and species of Aleyrodidae, comprising the following: *Trialeurodes coryli*, *T. packardi*, *T. morrilli*, *Tetraleurodes mori*, *Aleurochiton forbesii*, and also unidentified species from the common ragweed (*Ambrosia artemisifolia*), wild lettuce (*Lactuca canadensis*), *Viburnum acerifolium*, and *Eupatorium purpureum*. None of these, however, offered better conditions than are to be met with in *Trialeurodes vaporariorum*, and, on the whole, very little difference was observed.

I found it impossible to count the chromosomes in those phases which are undoubtedly spermatogonial. However, the mitoses are evidently normal. The equatorial plates of the various spermatogonial divisions observed are regular and identical in size.

The main problem of course centers in the question of how the haploid number of chromosomes is retained in the male during maturation. As is well known, Hymenoptera, in which parthenogenesis is haploid, simply eliminate the reduction. An attempt is indeed made to initiate the process, but the chromosomes do

not separate, and only a protoplasmic bud is constricted off as a result. The next and last division, then, has the nature of an equational division. We may probably conclude also in *T. vaporariorum* that the last division is equational. By analogy with the Hymenoptera, it should be the division preceding this last which is the critical one, but, apparently, there is nothing of exceptional character in the division concerned, for, as far as can be determined, division plates here are exactly like those found in the spermatogonia.

As in all other divisions, the most advantageous stage for counting the chromosomes is found just prior to the arrangement of the chromosomes into the equatorial plate. They are then irregularly distributed, but also less clumped, whereas they form a dense mass as soon as they have entered the plate. Eleven chromosomes can be made out. They are lengthened and identical in appearance with those observed in the somatic cells. Preparatory phases of this division show no trace of tetrad formation. Finally, the telophases show what are, in all probability, equal masses of chromatin in each daughter cell, and there is certainly no abortive division at this time. As indicated above, it is to all intents and purposes a spermatogonial division.

In spite of careful search, I could find no growth state preceding the final division, although it seems improbable that such a phase should be lacking. The daughter cells resulting from this division receive apparently equal masses of chromatin. The number of chromosomes prior to division cannot be made out with certainty, but must be very close to eleven, while the number after the division cannot be estimated. This, then, is probably the ordinary equational division of maturation.

It would appear, therefore, from the foregoing that there is in *T. vaporariorum* no abortive division, and that the first spermatocyte division has been entirely suppressed. This conclusion is indicated also in another way. The spermatogonial as well as the spermatocyte cells of the various stages are always assembled in definite groups, and in most cases show a radiating arrangement around a common center which seems to indicate that they originate from a single cell. All the cells of any one group are at

practically the same stage of development, so that they can be easily distinguished from the cells of neighboring groups, which are generally at different stages. In addition, all except the earlier spermatogonia, clearly show cyst walls. Since the cell number is practically identical in all cysts which are at the same stage of development, it is possible to count the cells after each division, and thus determine whether a certain percentage is lost. If the number of spermatozoa is proportional to the number of spermatogonial cells, then it can justly be concluded that no abortive division has occurred and that all divisions have resulted in functional cells. That such is indeed the case seems to be pointed out by the following:

Spermatogonial cysts show 7 to 8, 13 to 16, and 28 to 33 cells.

Cysts with cells after the first of the last two divisions show 52 to 59 cells.

Cysts with cells after the final division or spermatids show 103 to 111.

Cysts with ripe spermatozoa (which are easily counted when the sperm bundle is cut at right angles) show 101 to 123.

These figures show, as is to be expected, that a few cells are lost during development. But, certainly, there is no such essential loss as in case of the ant *Camponotus* (Lams, '08), where each spermatocyte produces but two functional spermatozoa, or the bee, where only one is finally produced. The objection may be made that not all of the spermatozoa of *T. vaporariorum* may be functional, and a large percentage may be rudimentary—assuming that the numerical proof given is the only evidence available. The only answer that can be made here is that spermatids and spermatozoa, respectively, show no differences of size or structure.

Comparing the phenomena here described with those seen in the nearly related groups of Aphids and Phylloxera, it will be seen that in those insects the chromosomes of the somatic cells have a very characteristic oblong or else lengthened shape. With a few exceptions, this form is lost in the maturation divisions, where the chromosomes become rounded and lumplike. This is true of both sexes. Exactly the same feature is noticed in the female of *T. vaporariorum*, the chromosomes of which seem to

follow the same transformations witnessed in the other groups mentioned. In contrast to this, the male, which in its somatic cells has chromosomes shaped like those found in the female somatic cells, shows no corresponding change in the maturation phases. The lengthened form is retained through all the divisions. This may have no significance, though it does seem to indicate an exceptional condition.

Judging from the evidence at hand, it seems probable that the reduction division has been eliminated altogether. It may be suggested that the division preceding the final one is also equational, and that since there is basically no difference between a spermatogonial and an equation division, we are concerned merely with a question of names. But our present knowledge of the meaning and mechanism of the ordinary equation maturation divisions is not sufficient to make such a statement altogether safe. Furthermore, the assumption of two equation divisions in maturation meets with serious difficulties arising from our present conceptions of the significance of maturation—a point which will be discussed at the end of this paper.

THE OCCURRENCE OF *TRIALEURODES VAPORARIORUM* IN ENGLAND

As I have noted in the introduction, although Williams apparently assumes that normal females of the English line produce both sexes when fertilized, such a fact remains to be experimentally established. Those of his breeding experiments which proved successful were made with the exceptional Merton stock which resembles the American line in its sex-ratios, and is therefore not of the true or normal English type.

The parthenogenetic production of females can be explained by an omitted reduction division, a reunion of one of the polar bodies with the egg-nucleus, or a doubling of chromosomes at some stage of development. So far as we know, the sperm nucleus of animals does not attempt union with the nucleus of the egg until the latter has undergone the maturation process. I think therefore, that it is only fair to assume that the spermatozoon may very well enter the egg of mated females of the English race, but that it plays no further rôle unless reduction takes place.

The fact that males do sometimes occur in English collections may mean that a few representatives of the so-called American race exist here and there or else that there is at times an irregularity or reversion to normality in the maturation divisions.

Should it be shown definitely that the English line does indeed produce a mixture of sexes from the mated females, we would have to take recourse to an explanation based on some such chromosomal behavior as is known in the Hemiptera and Orthoptera. To explain it on the basis of the cytological evidence from the American line or on the basis of the chromosomal behavior as found in the Hymenoptera, we would have to resort to the somewhat far-fetched hypothesis that there are two kind of spermatozoa, both of which, on entering eggs, cause them to undergo reduction, one of these spermatozoa being otherwise non-functional, so that the egg fertilized by it is left with the haploid number and produces a male. If the other kind of spermatozoon then be assumed to be normal and to restore the diploid condition in the egg it has entered, such an egg would produce a female. An analogous case, where the spermatozoon enters the egg, starts development, but plays no further part, and finally disintegrates, is found in *Rhabditis aberrans* (Krüger, '13).

The origin of the English race—the American line representing the normal or parent stock (Williams, '17)—may be due to environmental influence. It is conceivable that new physical influences of a hitherto unoccupied region may in some way act on the egg so as to modify or suppress the polar-body formation. But the question immediately arises why these same influences should not also act on the eggs of such a strain as was found at Merton and which was evidently not of the English type.

A more acceptable hypothesis would be based on an origin by mutation. Reciprocal crosses between the English and American lines should make it possible to determine whether the problem rests on a Mendelian basis. Once a female-producing line had become established, it might, and probably would, displace a male-producing line, as has been pointed out by Williams ('17) in some mathematical considerations of the problem. This would be especially true in a new country where the distribution

of the animal is limited to a small area at first. Crowding out of one line by the other would progress very fast in that case.

SEX RATIOS

Knowing that unfertilized eggs give rise to males in the American race, it is to be expected that, in a random collection, there would be a preponderance of that sex. Such is not the case, however, and this is due, no doubt, as Williams has already pointed out, to a difference in the duration of life of the two sexes. In one of my breeding experiments the female lived twenty-five days, while the males were generally dead by the tenth or eleventh day. Hargreaves ('14) mentions an adult that lived thirty-eight days, but does not give the sex of this specimen. He notes further that the duration of life is to some extent dependent on temperature, cold weather "making them live more slowly."

Judging from the cytological evidence, the production of the two sexes seems not to be due to two kinds of spermatozoa, but to be dependent merely on fertilization or its omission. Like the earlier observers, I found that virgin females produced only males, eight broods ranging from eight to fifty-two individuals having been raised to confirm this evidence. Mated females produced both sexes, and the proportion of these was very variable. This irregularity in sex ratios is open to two possible explanations: either the female has control over the spermatheca and, reacting to some stimulus, permits each egg to be fertilized or not, as the case may be, or the spermatheca acts perfectly mechanically and every egg is normally fertilized until the supply of spermatozoa is exhausted. In either case, the sex-ratio would be liable to great variation.

To determine this, I mated virgin females and kept a careful account of their offspring. I found that the eggs first laid were also the first to hatch and develop to the adult stage. In doing this, I did not keep track of every egg laid—a physical impossibility—but only of the batches deposited on successive days. If the female has control over the spermatheca, it is probable that the offspring will belong to either sex from day to day, the first eggs giving rise to a mixture of sexes. If, on the other

hand, every egg is fertilized until the spermatozoa are exhausted, the first eggs should all produce females, and after a variable period, all eggs should result in males.

I experienced the same difficulties as Williams in his breeding experiments. The small size and peculiar delicacy of the adults causes the handling of isolated individuals to result in many accidents. As the results in the following table seem to indicate, the female can control fertilization, and probably some stimulus determines whether spermatozoa are liberated or not whenever an egg is laid.

Order of emergence on successive days

	I	II	III	IV	V	VI	VII	VIII
a	2♀, 1♂	7♀, 4♂	5♀, 16♂	3♂	1♂	2♂	3♂	1♂
b	1♀	17♀, 1♂	3♀	5♂	7♂			
c ¹	2♀	3♀						
d	3♀, 1♂	1♀, 2♂	5♀	5♀, 2♂	3♀, 1♂	4♀, 8♂	4♂	
e	2♀	1♂						
f	1♀, 4♂	2♀, 4♂	3♀, 1♂	3♀, 1♂	2♂	1♀		

¹ The mother died on the third day after starting to lay.

THE MYCETOMA CELLS

The pseudovitelline or mycetoma cells are easily seen in the living pupae as two yellow masses situated just posterior to the midline. From five to eight of these cells are taken into each egg before laying, but their function is problematical, for they never seem to be used in the nutrition of the embryo. Occasionally a duct evidently connecting the cell mass with the exterior can be made out in the sections, so that possibly they may have a secretory function. Buchner ('18) has called attention to these cells as hosts of symbiotic 'fungi,' and has traced out their history in some detail.

As I have mentioned previously, in these cells the number of chromosomes is found to be irregular. Generally there are from thirty to thirty-five chromosomes, the number being independent of the sex of the individual. Such irregularities have often been observed in similar cells of other animals, and mitosis without

cell division and other abnormalities are not uncommon in such structures.

A very striking feature observed at certain stages is the presence of very definite tubules interspersed through the cytoplasm. These may stand in some relation to the symbionts described by Buchner, although the latter apparently did not see them in the European species he examined. They are in some respects very similar to Holmgren's canals, but, unlike the latter never extend to the periphery of the cell. In division they are placed immediately around the spindle. Their distribution seems haphazard at this stage. After division they appear to break up into granules, only to be reformed immediately afterward, but I am not prepared to maintain that this phenomenon may not be due to fixation.

I made some attempts to determine the nature of these structures—whether mitochondrial or otherwise. The poor preservative action of osmic mixtures when used in connection with these cells eliminated the Benda stain at the outset. The mixture of formalin and potassium bichromate employed by Kopsch gave very good fixation at times, and, when used in conjunction with Kull's modification of the Altmann stain, gave some excellent preparations. Here, as in certain gland cells of *Myxine*, recently studied by Schreiner ('16), the nucleolus and the thread-like inclusions stain red, while the chromatic material is a faint green. Such a stain is not very specific, however, for, as Professor Wilson pointed out to me, the acid fuchsin is apt to stain any cytoplasmic inclusion, regardless of its nature. Janus green used on fresh material did not stain the structures in question, but such negative evidence does not carry much weight. The tubules stained a deep black with haematoxylin. When fixed with the Gilson-Carnoy fluid, part of the tubule or what may be called the matrix disappeared, leaving a spiral skeleton or framework which retained the haematoxylin stain and still showed the tubular structure. Such pictures give the impression that they are composed of two different substances, only one of which is dissolved by the action of the strong fixing agent.

Schreiner's description of the origin of cytoplasmic granules in case of *Myxine* finds one curious parallel in these cells. Very often a cell will show three or more nucleoli, which, judging from their size, have originated from the normal single nucleolus. In many cells the nucleolus is seen dividing, and in others a sort of budding is observable. In the latter case, smaller masses of nucleolar material are visible in the nucleus, some just forming from the parent body, others separated from it by variable distances, but still connected with it through a faint but definite thread, and still others near the periphery of the nucleus and entirely independent of the nucleolus. I was unable to find such bodies still connected with the nucleolus, but outside of the nuclear membrane. In every instance where such an interpretation might have been made, the alternative of incorrect focusing or confusion with cytoplasmic inclusions of a different nature could be held. To eliminate the latter possibility, I tried the expedient of destaining very strongly. Since the nucleolar material retains the hematoxylin after the other cell constituents have become decolorized, its presence outside of the nucleus could then be determined with more certainty. But although the nucleolar material appeared perfectly sharp and distinct inside of the nucleus in such cases, none of it could ever be found definitely outside.

DISCUSSION

Without attempting to give here any general review of the great literature relating to parthenogenesis, I will briefly indicate certain difficult and still obscure points which seem to call for further investigation. It must be remembered throughout that parthenogenetic eggs belong to either of two groups—one in which they are incapable of fertilization and development is asexual in the true sense; the other in which the eggs may be fertilized or not, development proceeding in either case. Usually the unfertilized eggs of this last group then develop with the haploid number of chromosomes.

The researches of Schleip ('08), Nachtsheim ('13), and others have now well established the fact that in the majority of Hymen-

optera the sexual eggs normally undergo two maturation divisions, in the course of which the chromosome number is halved. As in case of *Trialeurodes vaporariorum*, such eggs will develop into males if unfertilized, but if the diploid number is restored through the entrance of a spermatozoon, a female is developed.

Although haploid parthenogenesis of the sexual eggs thus appears to be normal among Hymenoptera, there appear to be cases where certain eggs are asexual, parthenogenetic development taking place with the complete diploid number of chromosomes. As might be expected, such eggs give rise to females. This is found most generally in species where there is an alternation of sexual and parthenogenetic generations. I need mention only the gall-fly, *Neuroterus lenticularis* (Doncaster, '10, '11, '16), where the spring generation consists solely of parthenogenetic females, while the summer generation is represented by males and sexual females. The eggs of the latter undergo maturation and reduction like those of ordinary Hymenoptera, and since these eggs must be fertilized in order to develop, they always give rise to females (the parthenogenetic females mentioned above). The parthenogenetic females, however, produce two types of eggs. Those which give rise to the males again conform to the normal in that reduction takes place and parthenogenesis is haploid, while in those eggs from which females are developed, the diploid number is retained. Doncaster believes that there no polar bodies are given off. These proceedings are thus apparently clear, but what seems to be a more exceptional case is furnished by *Rhodites rosae*. No males have been found in this species by cytological investigators, and parthenogenesis is without doubt obligatory. Judging from analogous cases, it might be expected that such parthenogenesis is diploid, but analysis of the case presents various difficulties. Henking ('92) and Schleip ('09), both of whom have investigated maturation in these eggs, agree that two polar bodies are given off. Henking regards meiosis as normal, the chromosomes being reduced to the haploid number. The diploid number is, however, then restored in the first cleavage through a doubling of each chromosome.

If this account be correct, the case presents no difficulties, but Schleip states that he could find no such doubling of chromosomes after maturation. He believes that the chromosome number observed in the maturation is diploid and that this is kept constant because both maturation divisions are equational.

Such an explanation meets with serious difficulties, arising both *à priori* and from detailed observations on the meiotic phenomena, especially in case of the Hemiptera and Orthoptera. It has been established to a practical certainty that the reduction and equation divisions are perfectly definite in their operation. A fine substantiation of this rule is furnished, for instance, by *Trimerotropis*, an Orthopteron in which Miss Carothers ('17) has described several heteromorphic chromosome pairs, in which the synaptic mates differ distinctly in form. No correlation exists between the different pairs in regard to their distribution during reduction, so that a daughter cell may receive either member of such pair as a matter of chance. After separation, each homologue undergoes an equation division, and there is never a violation of this proceeding. A more special case is found in the 'm' supernumerary of *Metapodius* (Wilson, '09) and also the extra chromosome of *Oenothera lutea* (Gates, '14). Such supernumerary chromosomes may divide at either the heterotypic or homotypic division, but they never undergo more than one division. Finally might be mentioned the sex chromosomes, which are subject to the same rule. Before basing an explanation on the grounds of two equation divisions, therefore, a more sufficient proof than is given for *Rhodites rosae* and a few similar cases must be presented.

It is very possible that the problem involves the formation of multiple chromosomes. It was this most difficult point in Hymenopteran cytology which lay at the bottom of the long controversy concerning the bee. Nachtsheim ('13), who seems to have said the final word in this matter, offers the following explanation: The diploid number in the bee is 32, but through a coupling of these only 16 appear to be present in the oogonia. The reduced nucleus presents 8, and these must therefore be considered as bivalent. The case is complicated still further by the fact that 64 chromosomes appear in some somatic cells.

It is conceivable, therefore, that in *Rhodites*, also, there is at some stage a multiplication of chromosomes which restores the diploid number in the reduced nucleus. Perhaps this may occur in the oogonia, where Schleip seems to have made no exact counts. In one of the maturation divisions, the products of such a multiplication which have joined in pairs are separated from each other. This would then be a reduction division in the sense that paired whole chromosomes are separated from each other. The other division is then purely equational. Schleip's own observations show that phenomena such as have been described for the bee very probably do occur in *Rhodites* also, for he describes a pairing of chromosomes in the blastoderm nuclei, through which their number is temporarily halved. Furthermore, his figures of the first polar division show formations that may very well be tetrads, and do not speak at all against a reduction division.

Two equation divisions were also reported in the parthenogenetic eggs of the saw-fly, *Nematus ribesii*, by Doncaster ('07), but this interpretation has seemingly been abandoned, for Doncaster makes no mention of it in his "Determination of Sex" ('14).

Occasionally there are reports of cases which seem to contradict the general rule of chromosomal behavior among the Hymenoptera. Thus G. W. Onions (Jack, '16) found that virgin workers of the Cape honey-bee gave rise not only to drones, but also to workers and queens. Such cases are only evidence for the fact that irregularities are apt to occur in the polar body formation, and that some races and species are more prone to such irregularities than others. Suppression of reduction or reunion of polar bodies with the nuclei offers the most plausible explanation in such cases.

The manner in which the males of the Hymenoptera retain their haploid complex of chromosomes during maturation is too well known to merit a long discussion. They all agree in having an abortive reduction division, which does not result in changing the chromosomal constitution. As regards the equation division, there seem to be two natural groups. In one, including *Apis* (Mark and Copeland, '06; Meves, '07), *Xylocopa*

(Granata, '09), and *Osmia* (Armbruster, '13), the equation division results in two unequal second spermatocytes, only one of which forms a functional spermatozoon. In the other, comprising *Vespa* (Meves and Duesberg, '07), *Neuroterus* (Doncaster, '07), and *Camponotus* (Lams, '08), two equal cells result, both of which form functional spermatozoa. The rudimentary spermatozoon produced in the first group is evidently due to unequal division of extranuclear material, for in both groups the division of chromatin appears to be equal and is no doubt truly equational.

The rotifers almost certainly belong to the groups in which parthenogenesis is haploid. Parthenogenetic eggs which give rise to other parthenogenetic females give off only one polar body and no reduction takes place. The sexual eggs give off two polar bodies and the chromosomes are reduced to the haploid number. If fertilized, the diploid number is restored and a female is produced; if not, the egg develops into a male with the haploid number (Whitney, '09). Exceptional conditions are met with in the spermatogenesis. Whitney ('17, '18) describes two kinds of spermatozoa in each male, one being large and motile, the other smaller and apparently non-motile, and these are present in the ratio of 2 to 1. He believes that both classes of spermatozoa contain chromatin, and concludes that the first maturation division gives rise to two cells, one of which forms the smaller spermatozoa without further division, while the other divides once more to produce two large motile spermatozoa. The latter class thus undergoes two divisions and, although Whitney does not commit himself definitely on this point, the conclusion once more presents the serious difficulty of two equation divisions, since males are haploid to start with. When based merely on Whitney's numerical data, such an explanation is very tempting, but no cytological evidence has yet been produced in its favor. The need for future study of the spermatogenesis is obvious. Taking Whitney's evidence as given, it is barely possible that the numerical ratio may result from the existence of two classes of spermatogonia, equal in numbers, but not necessarily externally distinguishable, one of which would give rise

to oligopyrene spermatozoa directly, while the other underwent an equational division and produced eupyrene spermatozoa. But such an explanation would rest on a very insecure basis, for the whole problem of abnormal sperm formation is still very much unsettled.

The breeding experiments of Hindle ('17) with lice, *Pediculus humanus*, may be also mentioned here. Hindle obtained from single females broods consisting in some cases of both sexes, in others of males or females exclusively. Males were always put together with the females, but copulation was not observed, Hindle evidently assuming that this always took place. It seems to me that this is a fairly clear case of haploid parthenogenesis.² The fact that the same female in one instance produced consecutive broods of all males and all females seems to point to fertilization as the determining factor. Very probably the amount of sperm in the spermatheca determines whether females exclusively or a mixture of sexes is produced, while the entire absence of spermatozoa results in the production of males. *Anthothrips verbasci*, in which A. F. Shull ('17) has found both sexes produced by fertilized females, and males only by virgins, no doubt also comes under the heading of haploid parthenogenesis.

There are some cases where sexual eggs, capable of parthenogenesis, apparently develop with the diploid number of chromosomes if unfertilized. The tick, *Ambylomma dissimile*, which is parasitic on Amphibia and reptiles, gives rise to females through parthenogenesis, while fertilized females produce both sexes (Bodkin, '18). The numbers given are too small to admit of any definite conclusion, but very probably parthenogenesis is diploid, and in fertilized eggs the spermatozoon influences polar body formation in some way.

Very similar cases to this are found among the Orthoptera, a group in which the normal chromosomal behavior in the causation of sex is well known, females being homozygous and males

² Since the time of writing, K. Foote, *Bio. Bull.*, vol. 37, and L. Doncaster and H. G. Gannon, *Q. J. M. S.*, vol. 64, have investigated the cytology of *Pediculus*. Indications of a rudimentary division in spermatogenesis seem to confirm the above, but the chromosome counts are inconclusive.

heterozygous for sex. While reproduction is sexual in the great majority of the order, there are some well-established cases of parthenogenesis on record. Thus *Saga serrata* is represented by both males and females in Asia Minor, but in southern Spain only females are ever found (Bolivar, '97). Hebard ('18) has not seen a single male among some thousand immature and adult specimens of the blattid, *Pycnoscelus surinamensis*, although a male has recently been reported by another entomologist. MacBride and Jackson ('15) raised three thousand offspring of the phasmid, *Carausius morosus*, all of which were parthenogenetically produced, and among this number observed only six males and one gynandromorph. These and many similar cases demonstrate that parthenogenesis occurs, that in some species it is practically obligatory, and that with very rare exceptions it gives rise to females. A few cases are, however, known in which asexual reproduction is facultative, and eggs will develop whether fertilized or not. As in the obligatory type, parthenogenesis here produces females, while fertilized eggs give a mixture of males and females. I need mention only the grouse locust, *Apotettix*, reported by Nabours ('19), and the phasmid, *Clitumnus* sp?, which was studied by Fryer ('13). Experimental genetic work was done in both cases, and this showed that segregation occurred in the first generation of parthenogenetically produced offspring. This is good evidence for the conclusion that reduction occurs in these unfertilized eggs as well as in the fertilized ones. A further confirmation of this was obtained when Nabours found that the F_2 parthenogenetic generation was homozygous for certain characters under observation.

Cytologically, the maturation phenomena in parthenogenetic eggs have been studied only in *Bacillus rossii*, where von Baehr ('07) found two maturation divisions. He seems to believe that both of these are equational divisions (Buchner, ('16), has also gathered this impression from von Baehr's account), and that therefore no reduction takes place. However, his figures of equational plates of the first division show tetrad-like structures, and since he is unable to count or even estimate the oogonial or somatic chromosomes, it is probable that here also reduction occurs as in sexual species.

To sum up, the eggs of all Orthoptera are subjected to reduction. Those which are parthenogenetically developed give rise to females, while those which are fertilized result in a mixture of sexes. How, then, is the chromosome number kept constant, and why, since a reduced egg has only one sex chromosome, is not a male developed from such eggs? As indicated in case of the tick, this question might find a satisfactory answer in the assumption that the spermatozoon affects the formation or behavior of the polar bodies, such that its entrance into the egg causes the second polar body to be cast out or disintegrate, sex being then dependent on the kind of spermatozoon which has entered. We might then assume that in eggs that are not fertilized, the second polar body fails to be extruded or, if formed, it rejoins the nucleus. In the former case, there would be a haploid complex of dyads, each of which breaks up into the component units at a later stage, while in the last-named case, the diploid number is immediately restored by the rejoining second polar body.

Parthenogenesis is also known in the Lepidoptera, and in this connection the Bombycidae and *Liparis dispar* might be specially mentioned. Platner ('88) thinks that two polar bodies are given off by parthenogenetic eggs of the latter, but unfortunately he was unable to rear offspring from these to the stage where sex can be determined, and previous experiments with the same species admit of no definite conclusion. Although cytological work on parthenogenesis has been followed out to only a very limited extent, some very interesting observations have been made on the maturation phenomena in hybrids, which seem to offer the most convincing evidence thus far produced of the occurrence of two equational divisions in maturation. Federley ('13), in his well-known crosses of *Pygaera* species, found that no synapsis takes place prior to maturation and, from both cytological observations as well as the fact that after two maturation divisions the number of chromosomes is still practically equal to the sum of the haploid numbers of the parents, concludes that two equational divisions must occur. The evidence given by Fed-

erley is so clear cut and definite that it seems as if this interpretation is the only one that can be made at the present time. Harrison and Doncaster ('14), who crossed *Biston zonaria* with *B. hirtaria*, found that in the hybrids synapsis occurs to a limited extent. Pairing is also noticeable prior to the first maturation division, and such chromosome pairs undergo reduction in a normal manner. The unpaired chromosomes may either divide in each of the two divisions or else go undivided to either pole in one and divide equationally in the other division. The latter thus behaves, as might be expected, like an unpaired chromosome in the spermatogenesis of typical Hemiptera or in the lata type of *Oenothera* (Gates, '14). This, however, does not apply to those chromosomes which seemingly are subject to two equation divisions like those of the *Pygaera* hybrids.

Doncaster, observing that the *hirtaria* chromosomes are about four times as large as those of *zonaria*, but that the latter has 56 compared with 14 in *hirtaria*, suggests that the smaller number arises from the larger by a union of chromosomes. In other words, they are compound or multiple chromosomes. If such multiples be made up of unit chromosomes which have the same constitution (and they must bear some such relation to each other if the same ones always join to form the multiples), then in the 56 units of *zonaria* there must be 14 quartets of similar units. It is thus possible that the *Pygaera* chromosomes are really multiple chromosomes which in one of the divisions are merely separated into their component parts. This would then not be a true equation division, for it involves the separation of joined whole chromosomes, neither would it be a true reduction division, as I have mentioned under *Rhodites*. Nevertheless, some such process must take place in all cases where the diploid number of a reduced egg is restored through a reunion of the equational polar body with the nucleus or by a secondary doubling of chromosomes within a cell. All such chromosome sets must be made up of pairs of homologous units. How such multiple chromosomes manage to persist in the maturation of ordinary non-hybrid *Pygaeras* presents still another difficulty, but

this same difficulty exists also in case of the bee where the dyads likewise seem to persist through the maturation.

A few words might be said of the cytological work that Rosenberg ('17) has done on hybrids of the plant genus *Hieracium*. The maturation divisions are in most cases characterized by more or less irregularity. According to Rosenberg, a few of the unpaired chromosomes may undergo two equational divisions, while others are divided only once. But the very irregularity in the amount of pairing and the behavior of single chromosomes must make it practically impossible to be certain in these cases, except when none of the chromosomes are paired and the diploid number of chromosomes is found in each of the four resulting cells after two maturation divisions. Such a case, however, is not mentioned by Rosenberg.

Very puzzling conditions are met with in case of *Dinophilus*. Shearer ('12) described an entrance of the spermatozoa into the oogonia without a fusion of the nuclei. At one of the later divisions he assumed that the male nucleus did not divide with the female nucleus, so that the two daughter cells in one case have a biparental complement of chromatin, in the other a maternal only, the former becoming a female and the latter a male. Nachtsheim ('14), who recently investigated these conditions, finds that Shearer's interpretations are erroneous to a large extent. Thus, there is no precocious entrance of spermatozoa into the oogonia, and unfertilized females may contain female-producing eggs. Instead of the peculiar phenomena described by Shearer, there is a fusion of oocytes, and the male and female eggs apparently differ only in the larger amount of yolk contained in the latter. Possibly the animal is a protandric hermaphrodite and the number of oocytes or nurse cells which join the ultimate germ cell determine whether the male or female state is evolved. How the chromosomal mechanism is correlated with this is by no means clear, especially since Nachtsheim and Shearer both concur in that maturation phenomena are apparently the same in both kinds of eggs (two polar bodies are given off and the diploid number of 20 is reduced to 10). Possibly here also the chro-

mosomes observed in one sex may have a different value from those observed in the other.

The maturation phenomena in the lower Crustacea have not as yet been fully cleared up, although a good deal of work has been done here. However, it seems to be a general rule that parthenogenetic eggs which give rise to parthenogenetic females form only one polar body with no reduction of chromosomes. An exception to this rule was discovered by Brauer ('94) in a small proportion of the parthenogenetic eggs of *Artemia salina*. There two polar bodies were given off and reduction took place, but the second polar body then rejoined the nucleus and restored the diploid number. In the course of later investigations, Fries ('09) did not confirm Brauer in this respect and is inclined to doubt it, while Petrunkevitch ('02) regards the phenomenon as pathological. It is interesting to note in this connection that Artom ('11) has since found that there are two distinct races of *Artemia salina* in Italy, one of which is always sexual, while the other can develop parthenogenetically. The former has eggs which give off two polar bodies and undergo reduction, while the latter form only one polar body with no accompanying reduction. It may be that contradictory results obtained by previous investigators are due to the fact that they were working on similar different races. If crosses could be made between the two races, some light might be thrown on this problem. The fact remains in any case that the diploid complex is retained in some way or other in parthenogenetic eggs which give rise to another parthenogenetic generation.

Sexual eggs give off two polar bodies and reduction occurs. The question of how parthenogenetic sexual eggs which give rise to females (if there really be such) differ from those which give rise to males is not known. From the fact that reduced eggs must be fertilized in order to develop, and that reduction is known to occur in the spermatogenesis of closely related species, it seems likely that the male is not haploid. The male-producing eggs probably extrude certain chromosomes, as do those of Aphids and Phylloxera, and in the spermatogenesis there is perhaps a

process which again duplicates that found at that stage in those groups, only one functional (female-producing) spermatozoon being evolved.

There is no need to enter into details in the well-known cases of *Phylloxera* and *Aphids* (v. Baehr, '09; Morgan, '09). Briefly, it might be said that diploid parthenogenesis occurs and only one polar body is given off. In the female eggs this single division is equational, but in the male eggs certain sex chromosomes are cast out entire, and reduction therefore occurs as far as they are concerned.

E. Krüger ('13) has described a case of semiparthenogenesis in the nematode, *Rhabditis aberrans*, in which the egg gives off a single polar body and the division is equational. The spermatozoon enters the egg and starts development, but it does not conjugate with the egg nucleus and finally degenerates. Practically all worms found are hermaphrodites, and males are extremely rare.

In the trematode, *Diplodiscus temporatus* (Cary, '09), parthenogenetic eggs arising in the sporocyst give off only one polar body and no reduction seems to occur, as might be expected. This, of course, is a case of obligatory parthenogenesis.

Finally, mention may be made of artificial parthenogenesis. The starfish egg can be stimulated to develop before the polar bodies have been given off. Maturation will proceed, but according to Buchner ('15) the second polar body rejoins the nucleus, and segmentation stages show the diploid number of chromosomes. Unfortunately, no larvae have been reared to the stage where sex could be determined. Annelid eggs, which are also capable of being stimulated before polar body formation, have likewise not been reared to the necessary stage.

The sea-urchin egg cannot thus be stimulated unless the polar bodies have already been given off. Development therefore proceeds with the haploid number, and Delage has succeeded in raising two such embryos to a stage where he could see that one was certainly a male and the other probably so. Such numbers are of course too small to admit of any definite conclusion.

The fact that development in the frog can be induced artificially has been known for some time (Guyer, '07; Bataillon, '11; Loeb and Bancroft, '13 and others). Without entering into the question as to the nature of such a stimulus, it might be noted that Loeb ('18) reports that he has raised two females and seven males to maturity from such eggs. One of the males examined showed that the number of chromosomes is very probably diploid and certainly not haploid. On this basis Loeb offers as one of several hypotheses that the male is homozygous for sex, while the female is heterozygous, possibly being haploid. This would not explain the production of both sexes, although chromosomal irregularities accentuated by the abnormal method of development may possibly account for this also. But such a hypothesis disregards Swingle's ('17) counts of chromosomes in *Rana pipiens*, which seem to show that the female has twenty-six chromosomes while the males have twenty-five.³

The maturation phenomena in the egg of *Trialeurodes vaporariorum* as it occurs in America probably belong to the group of which the bee is a typical representative, but unlike the latter, does not present complications due to the formation of multiple chromosomes. Conditions shown by the English form, involving the parthenogenetic production of females, very probably resemble the cases mentioned in this discussion, where the diploid number is retained through a reunion of polar body and nucleus or secondary doubling in soma or germ cells. Regarding the spermatogenesis, the entire elimination of the reduction division is not found in any other animal so far investigated, although very often paralleled in the maturation of eggs.

That the two extreme types of chromosomal behavior during maturation should be found within a single order, and even in such closely related families as the Aphidiidae and Aleyrodidae, lends force to the argument that the two types of maturation are not in reality as different as appearances indicate. In the bee type, the diploid female complex naturally has 2 X, while the

³ Loeb has since then raised more frogs that were parthenogenetically produced, and C. L. Parmenter, Jour. Gen. Physiol., vol. 2, who has investigated their cytology, finds that the chromosome number is clearly diploid.

male has 1 X, irrespective of whether the sex chromatin is carried in a single chromosome or not. As far as this chromatin is concerned, such a case does not differ from that of a typical hemipteron with an unmated sex chromosome. No evidence is available that there is in these cases a distinct and separate X element, but in this connection it might be pointed out that coupling of this chromosome with a certain autosome has been observed in a number of cases (Sinety, in *Leptynia*, '02; McClung, in *Orthoptera*, '05; Boveri, '09, and Frolowa, '12, in *Ascaris*, etc.) Neither is the sex chromosome necessarily a single element, as Payne ('12) has pointed out especially in the reduviids. It is thus very possible that each autosome carries a certain amount of sex chromatin. The fact that in the diploid complex the autosomes are doubled as well as the sex chromosomes, if such a condition prevails, and that therefore the proportional relation remains unaltered, may indicate only that the two kinds of chromatin are independent of each other as far as influence on development is concerned. This does not imply that development could proceed in the absence of either of the two kinds of chromatin. It is probable that the autosomal chromatin is active in the causation of sex as well as in the development of all other characters, but this effect is irrespective of its amount involved. At a certain point, however, a decisive factor appears in the influence of the sex chromatin, and this influence, given the conditions already established by the autosomal chromatin, appears to depend on its quantity.

SUMMARY

1. *Trialeurodes vaporariorum* has two races which differ in their parthenogenetic behavior in that the English race gives rise to females and the American to males.
2. In the American race all eggs give off two polar bodies and undergo reduction.
3. Eggs that are not fertilized develop with the haploid number of chromosomes and produce males. Those which are fertilized regain the diploid complex and give rise to females.

4. In the spermatogenesis the haploid complex is retained probably through the entire elimination of the reduction division, and only an equational division occurs.

5. Fertilization of eggs in mated females is apparently under the control of such females, and dependent on some stimulus to which they may be subjected.

6. Discussion of cell inclusions in the Mycetoma.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Early prophase of first maturation division.
- 2 Tetrads.
- 3 Typical plate of first maturation, with eleven condensed tetrads.
- 4 Polar view of the anaphase of the first division.
- 5 Anaphase of the first division.
- 6 Plate of second polar division, with first polar body at the periphery.

Figures 26 to 29 and 36 drawn with no. 6 eyepiece and 1.5-mm. objective.
All others drawn with no. 8 eyepiece and 1.5-mm. objective.

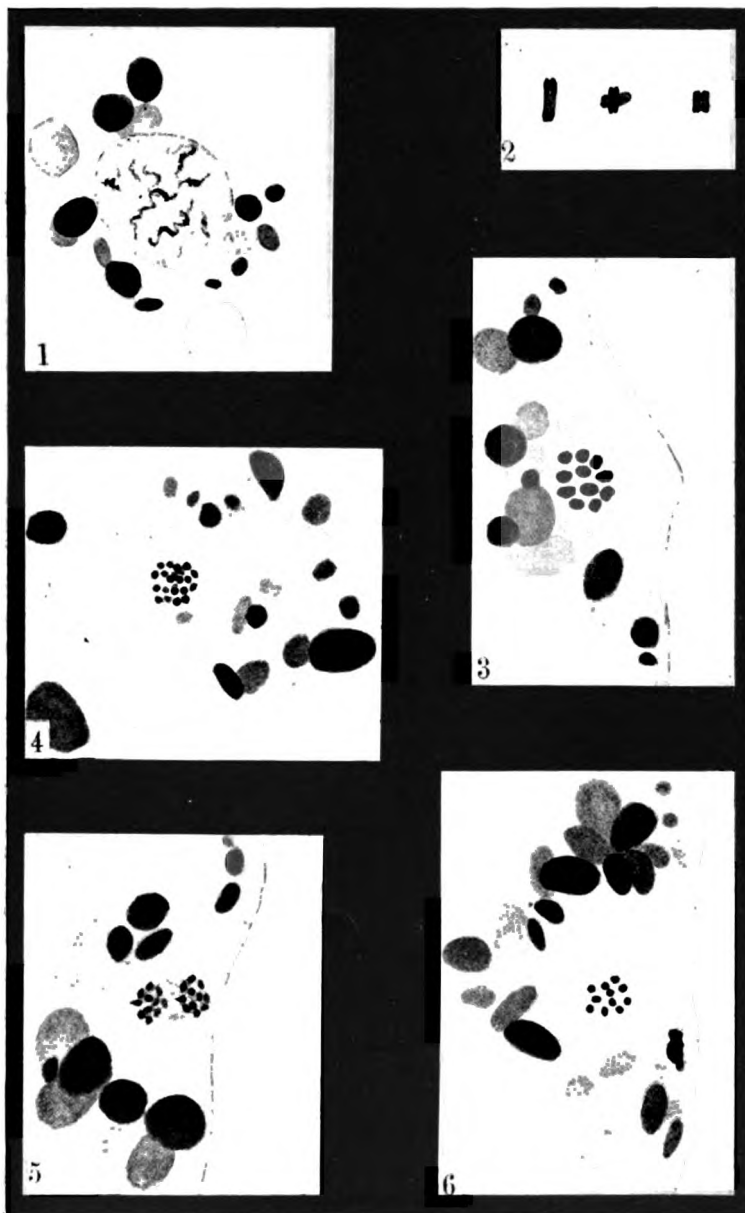


PLATE 2

EXPLANATION OF FIGURES

7 Second polar division in metaphase, with first polar body attempting to divide.

8 Nucleus of unfertilized egg in semiresting stage. Divided first polar body and the second polar body near periphery. (Two sections.)

9 Female pronucleus of a fertilized egg. Second and one of the first polar bodies near the periphery. Sperm head in the interior of the egg. (Two sections.)

10 Union of pronuclei (some chromosomes missing).

11 Fertilization nucleus with twenty-two chromosomes.

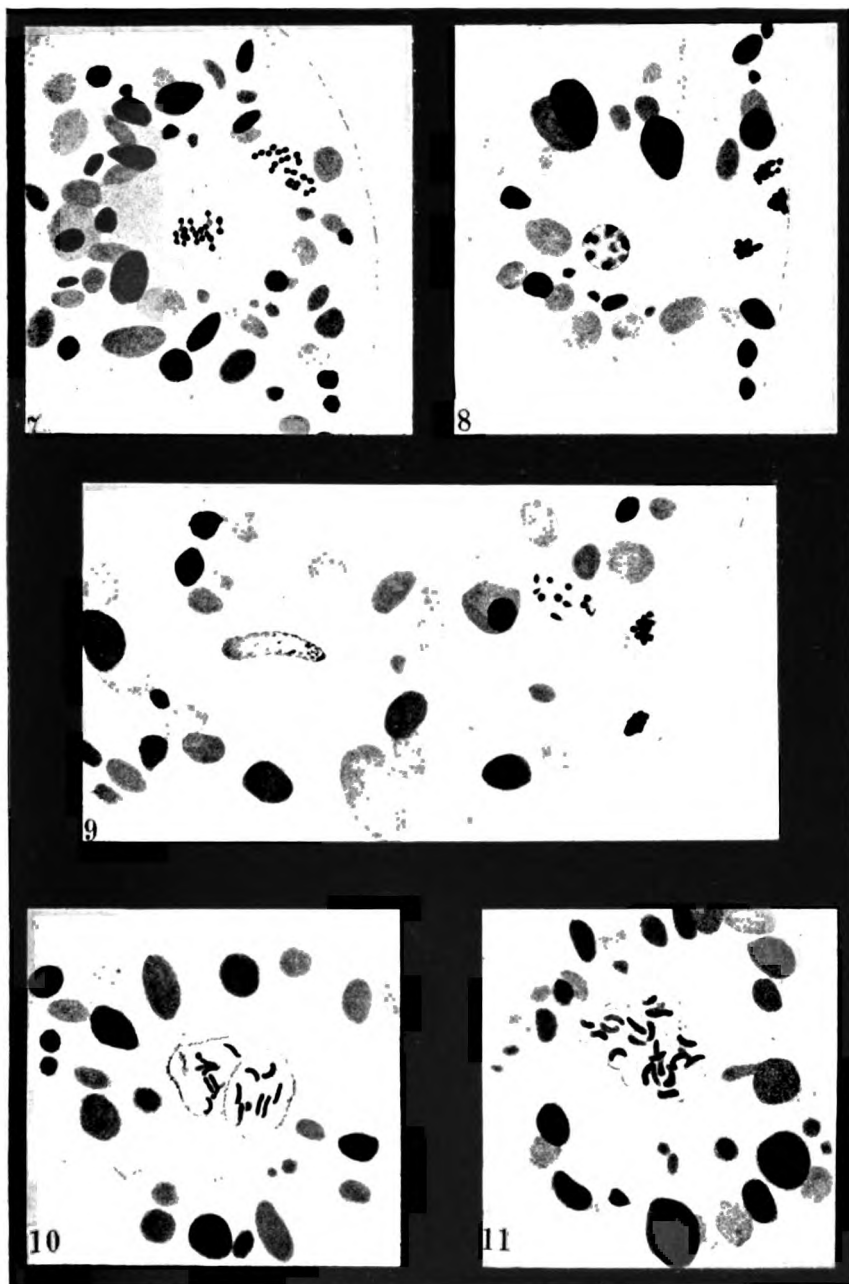


PLATE 3

EXPLANATION OF FIGURES

- 12 Nucleus of unfertilized egg, prior to first somatic division.
- 13 One of the first twelve somatic nuclei showing eleven chromosomes.
- 14 Cell of an early blastula, showing eleven chromosomes.
- 15 Cell in a gastrula stage, with eleven chromosomes.
- 16 Cell in a pupa that has testes developed.
- 17 Cell of an early blastula showing twenty-two chromosomes.
- 18 Cell of an early blastula showing twenty-two chromosomes.
- 19 Cell in a gastrula stage, with twenty-two chromosomes.
- 20 Cell in a pupa that has ovaries developed.
- 21 Division phase of early spermatogonia.
- 22 Division preceding the equational division, probably the last spermatogonial division.
- 23 The equational division.
- 24 Early spermatids.
- 25 Spermatid.

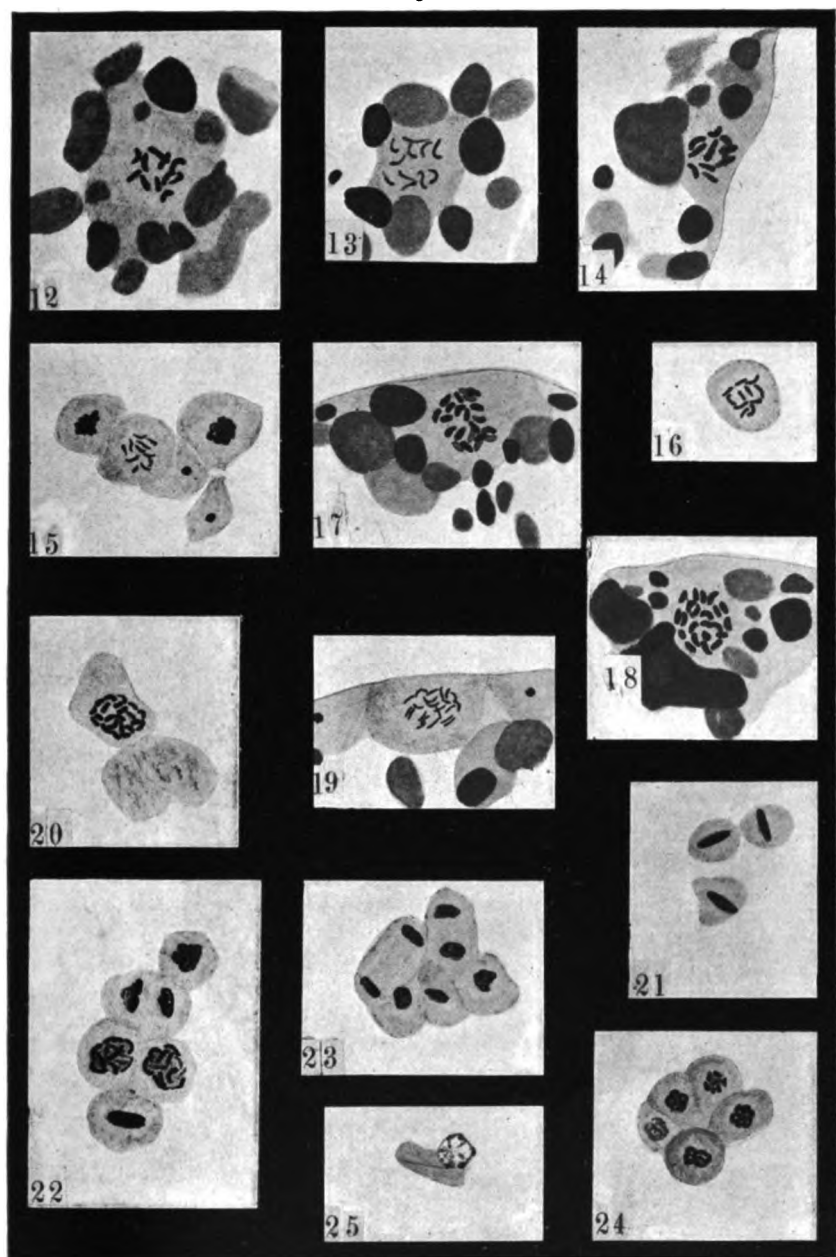
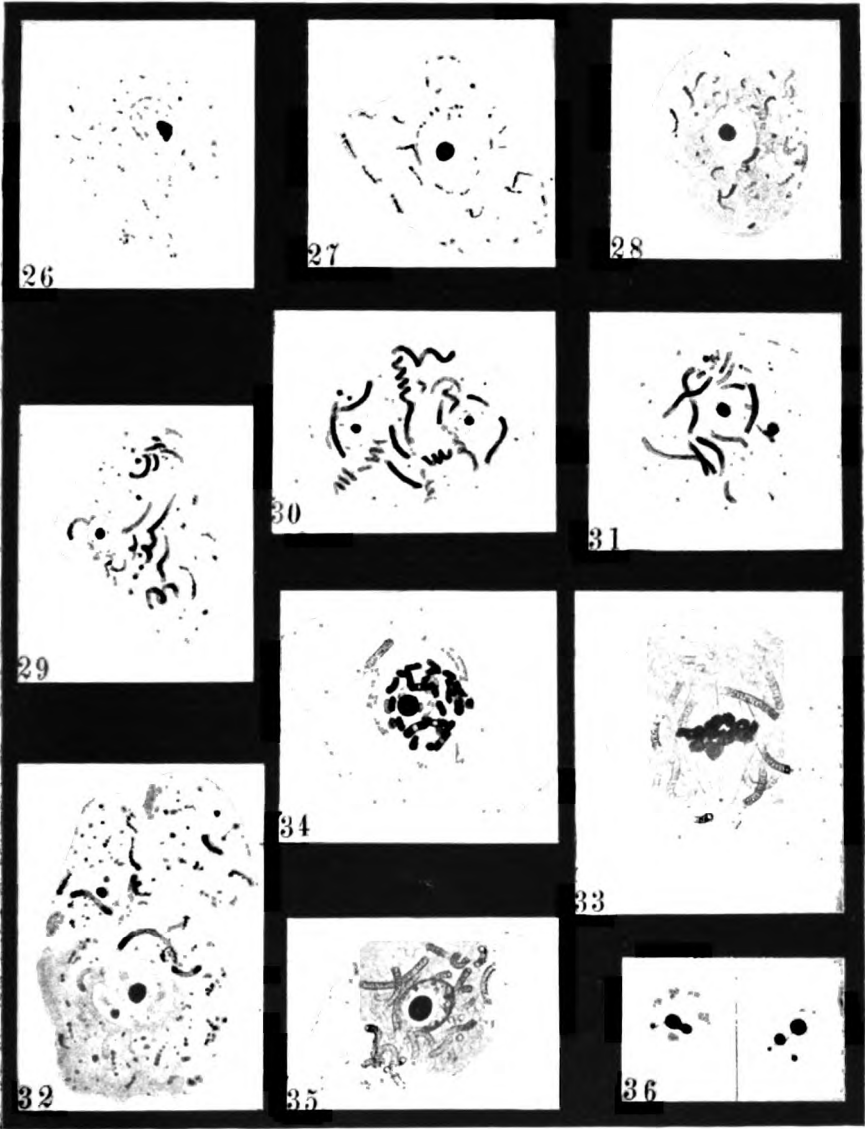


PLATE 4

EXPLANATION OF FIGURES

- 26 Mycetoma cell showing fine granules.
- 27 to 31 Series of stages showing formation of tubules.
- 32 Tubules breaking up.
- 33 Mycetoma cell in division, showing position of tubules at this stage.
- 34 Polar view of mycetoma cell in division.
- 35 Mycetoma cell in resting condition. (Figs. 33, 34, and 35 show effects of fixation with Gilson Carnoy fluid.)
- 36 Examples of budding from the nucleoli.



Resumen por el autor, Taku Komai.
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Espermatogénesis de *Squilla oratoria* De Haan.

Después de la última mitosis espermatogonial el material cromático se difunde por el núcleo; después aparecen filamentos leptoténicos separados y distintos, los cuales se disponen por parejas al llegar el estado de la sinapsis. Los números haploide y diploide de cromosomas son veinticuatro y cuarenta y ocho, respectivamente. En el citoplasma de la espermátida aparece una vacuola que aumentando de dimensiones llega a ocupar la mayor parte de la célula, de tal modo que el núcleo se hace completamente excéntrico. El núcleo pasa por un cambio bastante complicado, hasta que finalmente se convierte en una estructura rígida mente excéntrica. El núcleo pasa por un cambio bastante común y homogéneo. El centrosoma comienza a emigrar hacia el núcleo y termina por ponerse en contacto y encerrarse dentro de él, donde sufre una división y uno de los dos centrosomas hijos, que representa el centrosoma proximal, se transforma en un cuerpo en forma de bastón. El espermatozoide maduro es un corpúsculo esférico vesicular, y en uno de los polos de la esfera está situada la cabeza, que contiene dos centrosomas intranucleares.

Translation by José F. Nonides
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SPERMATOGENESIS OF *SQUILLA ORATORIA* DE HAAN

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FIFTY-ONE FIGURES (THREE PLATES)

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INTRODUCTION

The Stomatopoda has long been known to possess spermatozoa of a peculiar appearance, which differ widely not only from the ordinary filiform ones, but also from those usually found in the Decapoda. However, apart from a few rather old works, we have practically none in literature dealing with the morphology and genesis of the spermatozoon. At the suggestion of Doctor Yatsu, I undertook the study, the result of which has formed the data presented in this report. In pursuing the study as well as in working out the manuscript, I am deeply indebted to Doctor Yatsu.

LITERATURE CONCERNING THE STRUCTURE AND DEVELOPMENT
OF SPERMATOZOON OF THE STOMATOPODA

In a paper dealing with the structure of the reproductive organs of *Squilla mantis*, Grobben ('76) described for the first time the spermatozoon of the Stomatopoda. He says it is "kugelig, vielleicht etwas abgeplattet, von ganz homogene Aussehn mit sehr schmalem lichterem Band und 0.008-0.0085 Mm. im Durchmesser," and, further, that if the spermatozoon be treated with acetic acid, there appears a highly refringent body, elliptical when seen en face, which he thought to be the head of the spermatozoon.

Another work of the same author published two years later ('78), concerning chiefly the male reproductive organs of the Decapoda, contains scattered accounts about the structure of the testis of *Squilla mantis* and, further, a brief description of the development of the spermatozoon. The spermatid ('Samenzelle'), as described in that paper, has a spherical nucleus enclosing numerous nucleoli and surrounded by a faintly granular cytoplasm. At the commencement of transformation, the nuclear substance assembles toward one pole of the nucleus where the future head of the spermatozoon develops and takes a crescent shape. The substance next concentrates to form a hemispherical body which becomes homogeneous and refractive. Probably at the expense of the nuclear fluid, this body enlarges until it becomes spherical in shape and comes to lie at the center of the cell, whose cytoplasm also has now become homogeneous.

In 1885 Carnoy described the maturation divisions of *Squilla mantis*. He observed that the spireme is segmented into from twenty to twenty-four chromosomes, which arrange themselves in the equatorial plate with their longer axis parallel to the spindle axis. Each of the chromosomes splits longitudinally in the next division.

In the year following (1886), Gilson published a somewhat longer account of the spermatogenesis of *Squilla mantis* than that of Grobben referred to above. The testicular tubule is lined with a syncytial mass of cytoplasm which contains nuclei filled with clumps of chromatin. The mother-cells of the sper-

matozoon multiply exclusively by direct division. In the nucleus of those cells the spireme is formed, which is segmented into chromosomes. The sperm-cell undergoes almost no changes in external form until it becomes a mature spermatozoon. This latter process is initiated by the appearance of a small vacuole in the nucleus; this vacuole then enlarges considerably until it fills the greater part of the nuclear cavity, so that the nuclear substance comes to be pressed toward one pole of the nucleus against the nuclear membrane and assumes a cupule shape. The substance next concentrates, becomes homogeneous, and further continues to diminish in thickness for a while. Soon, however, the central part of the cupule-shaped body becomes inflated to take a button-shape and is brought into the cavity of the vacuole. In the meantime, the nucleus has a considerable growth, while the size of the cell remains nearly constant, so that it naturally follows that the cytoplasm is reduced a great deal. When, finally, the nucleus reaches full development, it fills the entire cell, the cytoplasm being completely lost, and the nuclear membrane fuses with the cell-membrane. The mature spermatozoon forms a vesicular corpuscle, spherical in shape and furnished with a very thick membrane. It contains a hyaline liquid, in which some plasmic remains may be recognized. At one pole of the wall of the vesicle the button-shaped body is attached; this generally is homogeneous, but may occasionally enclose a small vacuole. In the vas deferens the spermatozoa are agglutinated together by a cementing material into a firm cord.

More recently, ('09) Nichols described and figured the spermatozoon of *Squilla*, together with that of several other crustaceans. She says that the spermatozoon is "spherical in shape, the greater part of the cell consisting of a colorless vesicle filled with hyaline substance, while the only portion staining with methyl green is a button—or lens-shaped body at one pole of the sphere." She assumed the spermatozoon 'to forecast' that of the Decapoda.

MATERIAL AND METHODS

Squilla oratoria De Haan (*S. affinis* Berthold) is a stomatopod common along the Pacific coast of the milder districts of Japan. In the Gulf of Tokyo it is fished in great quantity throughout the year and forms a staple food. The material of the present study was obtained at intervals from November to May, 1917-'19. The males were all available for the study, since all of them supplied both early and advanced stages of spermatogenesis. To secure early stages, however, especially in spring months, it was necessary to take the hindmost region of the testis. For fixing, various reagents were tried, such as, 1) acetic alcohol; 2) acetic sublimate (glacial acetic acid in 3 per cent); 3) Bouin's fluid; 4) Worcester's fluid; 5) Flemming's strong solution, and, 6) Hermann's fluid. Of these the last two reagents were found to give the most satisfactory results and were used almost exclusively for the present study. Sections were cut from 4 to 10 μ in thickness. Smear preparations were fixed either with Bouin's fluid or with osmic vapor. For staining, Heidenhain's iron-hematoxylin was used, with or without counterstaining with orange G, Bordeaux red, Congo red, or acid fuchsin. Both safranin and Delafield's hematoxylin were found to be less satisfactory than iron-hematoxylin, and they were used only to check the above method.

MALE REPRODUCTIVE ORGAN OF *SQUILLA ORATORIA*

Grobben's description of the male reproductive organ of *Squilla mantis* ('76) applies very well to the same organ of *Squilla oratoria*. As was pointed out by that author, 'the organ is situated directly beneath the heart tube and above the digestive tract and hepatic organ, and consists of two distinct portions, namely, the gonad proper and the 'accessory gland.' The former lies in the posterior region of the body, ranging from the last thoracic somite to the telson, while the latter is located in the middle region, in the exposed thoracic somites. Aside from this distinction in situation, the two portions bear close resemblance with each other. Both form extremely slender

convoluted tubes, paired for the greater part of their length, only parts at the anterior extremity of the accessory gland and the posterior end of the gonad proper being unpaired. Starting from these unpaired parts, both the accessory gland and the gonad proper proceed in striking meandering courses, but largely along the longitudinal axis of the body, the former from anterior, while the latter from posterior, toward the last thoracic somite. At this somite, they both enter the penis, which is attached to the basal segment of the ambulatory leg. Somewhat before reaching the somite, the gonadial tube shows extreme convolutions, forming several complete loops in its course and presents an appearance of an entangled thread. Mature or nearly mature spermatozoa alone are contained in this part of the tube; it is consequently to be called vas deferens to distinguish it from the more posterior and less convoluted part, the testis in the strict sense. The accessory gland apparently has nothing to do with the production of spermatozoa; it contains neither mature spermatozoa nor any developmental stages of them.

ARRANGEMENT OF SEMINAL ELEMENTS IN TESTICULAR TUBE

As is shown in figure 1, the testicular tube is nearly round in cross-section and contains seminal cells representing various stages of development, save the central region where some vacant spaces are generally found. The cells are arranged in two or three distinct zones. In figure 1 three such zones are very clearly distinguishable: the outermost, made up of spermatocytes in synizesis stage dispersed with some spermatogonial and nutritive cells, another containing young spermatids, and still another with advanced spermatids. A similar arrangement exists throughout the whole length of the tube. It may be noticed that the nearer the anterior end, the older the cells are.

In a region of the outermost zone in the section of the testicular tube, perhaps along the middorsal line of the latter, is a cluster of cells (fig. 1, *pr.*) distinguishable from the neighboring parts by its characteristic appearance. It consists of a few large spermatogonial cells (*p.spg*) together with some nutritive cells (*nt*) and bulges out into the mass of seminal cells of more

advanced developmental stages. Without doubt, this cluster represents the proliferating region of the seminal tube.

A similar arrangement of seminal elements has been observed in crabs by Binford ('13) and Fasten ('18). However, in those cases, the regions containing younger elements appear in section crescentic in outline and restricted to one side of the tube wall, whereas, in *Squilla*, they form complete rings of nearly uniform thickness.

The vas deferens is lined with a rather high epithelium enclosing large nuclei. The spermatozoa contained in the cavity form a compact mass, apparently being agglutinated by some cementing material. A broad space is often found between this mass and the wall surrounding it.

SPERMATOGONIAL AND NUTRITIVE CELLS

The transformation stages of the seminal cells up to the production of spermatids have not been traced in detail, so that I can give but a brief account of them. The spermatogonial cell (figs. 2 and 3) is polygonal and is marked off from its neighbors by a fairly distinct cytoplasmic wall. It contains a large vesicular nucleus, which is usually spheroidal or ovoid. Numerous chromatin granules of varying size are found in it. A few largest ones, occurring in the central part of the nucleus, represent the karyosomes and forms the centers of radiating linin strands, along which are arranged smaller and less conspicuous granules.

In the cytoplasm and close to the nuclear membrane, are bodies apparently to be identified as the 'chromatoid bodies' (fig. 2, *k*). They may be spheroidal, dumb-bell- or spindle-shaped and stain in basic dyes as strongly as the chromatin granules in the nucleus. Some of them are often so large and conspicuous as to be easily detected under low magnification, while some may be extremely minute and barely discernible with very high power. Small spheroidal ones look more like a centrosome, and it is rather hard to distinguish the former from the latter. The centrosome (*c*) is a single minute spheroidal body lying close to the nuclear membrane. No idiozome could be detected around it.

As was pointed out by Binford ('13) and Fasten ('18), it is possible to distinguish primary and secondary spermatogonia, although the distinction between the two is not infrequently rather obscure. The former (fig. 3) are restricted to the proliferating region mentioned above, whereas the latter (fig. 2) are distributed throughout the remaining outermost zone of the testicular tube. Apart from this topographical distinction, the two kinds of spermatogonia differ in some structural details: the nucleus of the primary spermatogonia is larger and more vesicular than that of the secondary and the karyosomes of the former are more regularly spherical.

In the spermatogonial mitoses no continuous spireme is formed. The chromosomes *ab origine* are ovoid or ellipsoid in shape. In two fairly good equatorial plates (figs. 4 and 5) I was able to count forty-eight chromosomes. Between two adjoining chromosomes a linin strand is often detected. The chromatoid body (fig. 6, *k*) lies outside the spindle and enters one of the two daughter-cells without division.

Interspersed between the spermatogonial and spermatocyte cells are numerous nutritive cells (fig. 1, *nt*, fig. 7). They show usually some degree of resemblance in appearance to the spermatogonial cells, but may be distinguished by the fact that the nucleus stains somewhat darker by basic dyes and is usually irregular in shape: triangular, crescentic, sausage-shaped, or even with pseudopodia-like projections. In some cases the nucleus exhibits an appearance highly suggestive of the occurrence of amitosis. The boundary of the cell is often difficult to detect; furthermore, not infrequently a group (three to five) of nuclei are embedded in a common syncytial mass.

Among workers of decapod spermatogenesis, the origin of spermatogonial and nutritive cells has been a matter of great dispute (Fasten's paper, '14). As to *Squilla*, Grobben ('78) points out the occurrence of 'Ersatzkeim' and Gilson ('86) speaks of 'plasmodium pérphérique,' both undoubtedly referring to the nutritive cells. However, concerning the question of their origins, they do not express any definite opinion. I could likewise obtain no data to decide the question, but this much

may be said with fair certainty, that the two kinds of cells under consideration have a common origin, and that no nutritive cells transform into spermatogonial cells.

GROWTH PERIOD AND MATURATION MITOSES

The chromatin of the nucleus which has undergone the last spermatogonial mitosis diffuses so completely in the nuclear cavity that the entire nucleus stains almost uniformly grayish with iron-hematoxylin. Soon, however, leptotene threads make their appearance in it. They increase gradually in thickness and staining capacity, and develop into separate threads (figs. 8 and 9). They are so numerous and overlie one another so intricately that a correct count seems hardly possible. But roughly one may say that they are forty-eight, the above-mentioned diploid number. At first, they are distributed throughout the entire nuclear cavity; soon, however, they mass together in the center of the latter, leaving a wide space in the marginal region (fig. 10). In spite of the position of the threads, this stage in *Squilla* represents the synizesis stage. According to Nichols ('02, '09), the same appears to be the case with the corresponding stages of *Oniscus*, *Hippa*, *Talorchestia*, and *Idotea*. No nucleolus, however, is found in the present form, contrary to her observations on those forms.

At the climax of the synizesis, the individual chromatin threads can hardly be made out; but somewhat prior to this there is a period in which the disposition of the threads may be fairly clearly detected. Here the arrangement of the threads in parallel pairs is very apparent; several pairs, each consisting of two threads of nearly equal length, appear in the nuclear cavity (figs. 11 and 12). Thus it seems to be clear that we have before us 'parasynapsis' instead of 'telosynapsis.' The same is also true of *Cambarus* and *Cancer* (Fasten, '14, '18) and probably of *Idotea*, *Hippa*, and *Homarus* (Nichols, '09).

When synizesis is over and the nucleus enters into diakinesis, the chromatin threads, nearly twice as thick as those of the preceding stages, come into view. In material fixed with

Hermann's fluid, stained with iron-hematoxylin and extracted fairly sufficiently, a longitudinal split is very clearly observable in each thread (fig. 13). In the pachytene stage the threads often present a distinct bouquet-like arrangement, most of them in the form of a complete loop, being arranged around a center of grouping, where they fuse with each other (fig. 14). No accounts of such peculiar arrangement of pachytene threads are found in Fasten's two papers nor in Nichols's description of the synizesis of *Homarus* and *Hippa*. But the latter author's account ('09) of the corresponding stage of *Idotea* and *Talorchestia* apparently indicates the occurrence of such arrangement. Binford's figures 10 and 11 also show the similar feature.

As the nucleus passes from the leptotene to the pachytene stage, the size of both the nucleus and the entire cell increases considerably. In the pachytene stage, which lasts for a tolerably long time, the nucleus and the cell continue to grow in some measure.

I could not determine the precise procedure by which each pachytene thread is transformed into a tetrad; I shall accordingly confine myself to a few remarks on my figures 15 to 17, which I believe to exhibit some of the successive steps passed by the threads during the change in question. In figure 15 the nucleus contains several figures, many of which are in the shape of U or V, both arms consisting of two longitudinal halves. In the next figure (fig. 16), most of these U's and V's have been transformed into hollow squares, probably by opening out of the longitudinal halves of individual arms. Lastly, in figure 17, the nucleus encloses rings, crosses, rods, and the like, indicating that the formation of tetrads has been completed. Without question, every variety of the tetrads has arisen from those squares shown above. The above observation on the tetrad formation, fragmental as it is, suggests the occurrence of more complicate processes, such as are clearly demonstrated by Wilson ('12), Montgomery ('11), and Wenrich ('16) in the spermatogenesis of some insects.

All the tetrads soon condense into rod-, dumb-bell- or crescent-shaped chromosomes, which are located mostly in the superficial

part of the nuclear cavity (fig. 18). I could not determine whether or no there is such a regularity in the orientation of particular chromosomes as is maintained by Nichols ('06) in the cases of *Oniscus* and *Porcellio*. In the stage succeeding, the chromosomes travel toward the equatorial region of the nucleus, whose membrane in the meantime vanishes and the achromatic spindle-fibers make their appearance (fig. 21). In the polar view of the metaphase (figs. 19 and 20) a few chromosomes overlies some others. It needs hardly be mentioned that the chromosomes are very large as compared with those appearing in the spermatogonial mitoses. The reduced number of chromosomes is twenty-four. The daughter-nuclei produced pass directly into the succeeding division without entering into the resting stage. In the equatorial plate of the latter division twenty-four chromosomes again seem to be found, but I could not accurately determine the number; the chromosomes are considerably smaller than those occurring in the preceding division.

The chromatoid body is visible throughout the above successive stages, virtually without undergoing any perceptible change altogether. It lies usually close to the nuclear membrane, and when the spindle is formed, it is always situated outside of the latter. Varieties of form found in spermatogonial cells are also discernible.

TRANSFORMATION OF SPERMATID INTO SPERMATOZOON

At the end of the second maturation division, the chromosomes group themselves at the poles of the spindle so closely together that they form a compact chromatin mass (fig. 22). Soon, however, clear spaces make their appearance within the mass (fig. 23), which rapidly increase in size, until the entire nucleus comes to exhibit a reticular structure. This change of the nucleus is shown in figures 23 to 26. Around the reticular nucleus is often seen a clear space devoid of granulation (fig. 26, the cell on the left side). The chromatoid body is still present.

The nucleus maintains the reticular state for a fairly long time; then some of the meshes of the network coalesce and

undergo rapid development, to produce a single large mesh which presses the remaining part of the network against one pole of the nucleus into a crescent-shaped mass (fig. 27). This stage is very frequently met in the course of transformation of spermatid; in figure 1 the middle zone consists of this stage. Both Grobben ('78) and Gilson ('86) mention the occurrence of a stage in which nuclear material assembles at one side of the nucleus and assumes a crescent shape. This apparently refers to the stage just described.

When further change sets in, the chromatin substance begins to diffuse into the single large mesh, so that the latter diminishes gradually in size (figs. 28 to 30) until finally it disappears altogether and the nucleus comes to stain uniformly dark (fig. 31). During the progress of the change, the size of the entire nucleus diminishes somewhat, and a clear space develops around it. Soon, however, the nucleus enlarges, its consistency reduced, and its power of taking stains decreases, so that it comes to stain grayish in iron-hematoxylin (figs. 32 and 33). The successive transformation stages of the spermatid nucleus described above may be found in a single slide and is not very hard to follow. Similar changes were traced by Spitzchakoff ('09) in the spermatogenesis of *Leander*; thus my figures 27 and 28 apparently correspond with his 5 to 7, my figures 29 and 30 with his 8 and 9, and my 32 and 33 with his 10, respectively.

The spermatid (fig. 33) is now polygonal and contains a spheroidal nucleus at the center. The nucleus appears finely granular and has a rather loose consistency, its appearance somewhat reminding one of a ball of wool. The outline of the nucleus is often rather indistinctly defined. In cytoplasm is a mass with a granular appearance representing the 'mitochondrial body' (*m*) of authors on the spermatogenesis of the Decapoda. The body lies, in the majority of cases, where there is the greatest space between the nucleus and cytoplasmic wall, presenting frequently a crescent shape and stains with basic dyes usually somewhat more weakly than the nucleus. The cytoplasm further contains the chromatoid body (*k*), which has nearly the same appearance as seen in preceding stages. Usually only one

body occurs; rarely two larger ones and a few additional minute ones may be present. Around the body is a narrow clear space which is especially marked when the body is imbedded within the mitochondrial body. The centrosome (c) is also near the nucleus; it is a minute body with no idiozome-like structure around it.

The spermatid retains the above state for some time. Further transformation is initiated by a peculiar movement of the centrosome, which starts to wander toward the nucleus. It soon comes in contact with the nuclear membrane and further makes its way into the interior of the nucleus (fig. 34), until it is enclosed within the latter (fig. 35). The nuclear membrane seems to afford but slight hindrance to this movement of the centrosome and to give way very readily to it. The centrosome lies at the centre of a clear space in the nucleus, developed apparently by recession of the nuclear substance under the influence of the body.

While the above change is going on, a vacuole makes its appearance in the cytoplasm (fig. 36, v). This may occur somewhat before the centrosome becomes enclosed within the nucleus, more frequently, a little later than the latter change. Sometimes two or more vacuoles appear simultaneously (fig. 40), but soon they coalesce into a single one. The vacuole then enlarges markedly, until it occupies by far the greater part of the cell, so that the nucleus becomes quite eccentric in situation and takes a hemispherical shape (figs. 37 and 38). The mitochondrial body is located on the side opposite the vacuole and lies over the nucleus, assuming the shape of a cupule. The above change in the cytoplasm may be initiated by the appearance of a clear space along one side of the nucleus, which space enlarges subsequently to arrive at the same result. Meanwhile, the nucleus continues to be reduced in size, while its staining capacity is greatly intensified.

The centrosome now undergoes a noteworthy modification. The body, which has grown somewhat in size within the nucleus, is divided into equal halves (fig. 39). Both are imbedded in a common clear space (fig. 41), or each in its own space (figs. 40

and 42). Rarely, this division of the centrosome is completed before any trace of vacuole can be detected in the cytoplasm (fig. 41).

The nucleus next undergoes an important change, both in shape and in consistency. To this time the nucleus has been hemispherical in shape, granular in consistency, and stained very heavily with iron-hematoxylin quite dark, but it now becomes ovoid (figs. 43 and 44) and then hemispherical, but with its flat surface away from the center of the cell, contrary to the initial state (figs. 42, 47 to 51). It becomes, moreover, quite homogeneous in consistency and stains with iron-hematoxylin a tawny color, save the marginal part which is still dark (figs. 46, 49 to 51). This change of consistency advances from the peripheral part centrad, as is clearly shown in figure 43, in which the central region of the nucleus is still dark and granular, while the marginal part has already become homogeneous. The nucleus in the meantime comes into intimate contact with the cell wall adjacent to it, where a low conical prominence develops from the nucleus, presenting a granular appearance, especially in its basal part (figs. 44, 47 to 51 *p*). Without doubt, this prominence represents the perforatorium. But it is somewhat doubtful if it does develop actually from the nucleus, inasmuch as there is possibility of the cytoplasm taking part of its formation. Since the prominence arises where the nucleus is in intimate association with the cell wall, it is not easy to ascertain the fact definitely. For the present, after having gone over tolerably many preparations, I am inclined to claim the nuclear origin of it.

Just before this change of the nucleus, one of the two centrosomes elongates to assume a rod-shape and takes the position of the axis of the perforatorium (figs. 47 to 51, *c.1*). The clear space around that centrosome is not infrequently discernible after the transformation (figs. 49 and 50), but later it appears to fade away. Figures 44 and 45 illustrate the change of the centrosome just mentioned. In figure 44 the centrosome is in the course of transformation at the distal extremity of the nucleus, which is ovoid in shape; around the two centrosomes is a

clear space common to both. Next, in figure 45, the centrosome has already completed its transformation, while associating with its fellow and before being imbedded completely within the nucleus; such however, is apparently to be regarded as an abnormal case. Judging from what is represented by these two figures, but particularly by the latter, it is very evident that the centrosome converted into rod-shape is to be identified as the proximal centrosome and not the distal as in the decapod spermatozoon.

Contrary to the proximal centrosome, the distal one (c. 2) undergoes virtually no change at all. It is placed usually in the center of the homogeneous part of the nucleus (figs. 47 and 51), but may not infrequently be quite eccentric in position in that part or may be situated just upon the boundary between that part and the perforatorium (figs. 49 and 50), or even within the latter (fig. 48).

The nucleus further diminishes its size, whereas the entire cell enlarges and its wall thickens, until finally the mature state is attained (fig. 51).

By the stage shown in figure 39 the mitochondrial body forms a cupule-shaped mass lying over the nucleus; when the nucleus comes in contact with the cell walls it assumes a ring shape and takes the position between the nucleus and the vacuole in the cytoplasm. As the transformation of the spermatid proceeds, the body becomes less significant, and in the mature spermatozoon it is no longer visible. It is not at all clear what part the body contributes to the formation of the spermatozoon, save the fact that it seems not impossible that it might constitute a part of the wall of the cytoplasmic vesicle. The chromatoid body is very easily recognizable in almost all immature spermatozoa (figs. 47 to 50). It may lie where there is some remnant of cytoplasm, either in the region directly adjacent to the nucleus or very distant from it. This body likewise appears to play no important rôle whatsoever in the formation of the mature spermatozoon, and in the latter it is no longer visible. It is not clear whether the body degenerates in situ or is expelled to the exterior from the spermatozoon, as was observed by Wilson ('13) in *Pentatoma* and by Fasten ('18) in *Cancer*.

Figure 51 represents a mature spermatozoon. It is a spherical vesicular body and bears a lens-shaped nucleus, the head, at one pole of the sphere. The size of the spermatozoon is from 9 to 11μ in diameter, while the head measures from 4 to 4.5μ in transverse and 2.5μ in vertical diameters. The membrane of the vesicle is fairly thick and appears to be somewhat resistant against pressure. Its outer surface seems to be covered with some glutinous matter, since the mature spermatozoa forms a compact mass in the cavity of the vas deferens. The vesicle contains a hyaline substance which coagulates when fixed, and then presents a faintly granular appearance. The head consists of, 1) a main part, homogeneous in consistency; 2) a subordinate conical part of granular appearance, representing the perforatorium; 3) a rod-shaped body standing in the axis of the perforatorium and derived from the proximal centrosome, and, 4) the distal centrosome imbedded within a vacuole occurring in either parts 1 or 2, or on the boundary of these two. The head is highly refringent and appears very compact. It is, moreover, very resistant and hard to disintegrate, although often its shape changes to some extent by fixation. Both Gilson ('86) and Nichols ('09) recognized that the mature spermatozoon of *Squilla* is a spherical vesicular body, at one pole of which is attached the lens-shaped or button-like head. No accounts however, are to be found about the more minute structure of the head, especially that of the centrosome and the body derived from it.

GENERAL DISCUSSION

a. Synapsis

Recently, ample evidence has been accumulated in favor of the occurrence of parasynapsis, instead of telosynapsis, through cytological studies upon various forms of animals. Especially works by such authors as Grégoire ('10), Montgomery ('11), Wilson ('12), and Wenrich ('16) seem to have demonstrated in the clearest way the validity of that view. As also in higher crustaceans, Fasten ('14, '18) maintains parasynapsis, and his

evidence for it derived from his study of the spermatogenesis of *Cambarus* and *Cancer* seems to be fairly convincing. Likewise, in the present material I was able to trace fairly well the successive changes of chromatin threads from the leptotene to the pachytene stage, and, notwithstanding that my observation is regrettably imperfect as to the important phenomenon of the tetrad formation, there seems to be hardly any room for doubting the existence of parasynapsis for the present case.

b. Comparative study of spermatozoa of the Decapoda and the Stomatopoda

As has already been mentioned, Nichols ('09) has pointed out that the spermatozoon of *Squilla* "seems to forecast the spermatozoon of the Decapoda." It is true that the spermatozoon shows at first glance a marked resemblance to that commonly found in the Decapoda, especially in forms belonging to the group Reptantia. However, to decide the question how far this apparent resemblance has to do with affinity in a real morphological sense, a more careful study is necessary. On the morphology and development of the decapod spermatozoa much is known through works of Grobben ('78, '06), Gilson ('86), Brandes ('97), Labbé ('03, '04), Koltzoff ('03, '06), Andrews ('04), Nichols ('09), Retzius ('09), Binford ('13), Reinhard ('13), and Fasten ('14, '18). An excellent review of the literature occurs in Fasten's earlier work ('14).

The above resemblance is mainly due to the fact that the sperm tail is replaced by a cytoplasmic vesicle which gives the entire spermatozoon an appearance radically distinct from the ordinary filiform ones. The vesicle arises in both spermatozoa from a vacuole appearing in cytoplasm and enlarges to occupy by far the greater part of it. Thus far the vesicles occurring in the two kinds of spermatozoa are in agreement. In the decapod spermatozoa the vesicle contains a substance staining rather deeply with various dyes and, according to Koltzoff ('03, '06) and Reinhard ('13), it arises by the accumulation of a kind of granule called by them 'Kapsel- or Schwanzkörnchen.'

In the mature state of the spermatozoa the vesicle acquires a firm consistency, probably by changing its nature into chitin, and it appears refringent. Internally, it contains two compartments, namely, an outer and an inner, called by Fasten ('18) 'first or primary vesicle' and 'second or secondary vesicle,' respectively. In the *Squilla* spermatozoon, on the other hand, the vesicle is simple, not divided into compartments, and contains no centrosome; its enclosure takes almost no stain at all throughout all stages of the formation of the vesicle.

A no less important distinction is to be found in the head or nucleus. In the Decapoda it appears to have a rather loose consistency and often stains but weakly. Thus in the crab spermatozoon Binford ('13) has pointed out that it is rather difficult to distinguish the nucleus from the cytoplasm in which it is imbedded. This is in sharp contrast to the fact that in *Squilla* spermatozoon the nucleus acquires a consistency entirely different from that of the cytoplasm and is marked off very clearly from the latter. As a matter of fact, this consistency of the sperm head reminds one of that of the vesicle of a decapod spermatozoon. Moreover, the head encloses the bodies derived from the centrosome just as it is in the vesicle of the latter spermatozoon. Singularly enough, the head of the *Squilla* spermatozoon shares such important features with the vesicle of the decapod spermatozoa.

There remains to be considered the structures arising from the centrosome. Many authors on the spermatogenesis of the Decapoda claim that one of the two centrosomes produced by division of the original one becomes rod-shape to form the 'central body' within the secondary vesicle, while the other maintains that it retains its initial state at the base of its fellow. In the present material, too, one centrosome becomes rod-shape, while the other undergoes no perceptible change. The question naturally arises as to the homology of the two centrosomes of *Squilla* with those of the decapod spermatozoa. As has been shown above, in *Squilla* the centrosome which takes the rod shape is the one which is more approximate to the nucleus, while the other, remaining unmodified, is the distal centrosome

lying more apart from the nucleus. In the decapod spermatozoa, on the other hand, the centrosome undergoing transformation is invariably the distal one and that which does not, the proximal centrosome. In short, the resemblances of the spermatozoa of the two groups, striking as they are at first glance, seem in large measure to be of a rather superficial nature than of a strict morphological significance.

It may be added that a case somewhat suggesting the change of the proximal centrosome in the *Squilla* spermatozoon is afforded by the spermatogenesis of *Gammarus* (Köster, '09, '10). According to the accounts of that author, the proximal centrosome placed at the base of the sperm head sends out a fiber through the head toward the perforatorium and becomes united with the latter.

SUMMARY

1. In the testicular tube the seminal cells are arranged in two or three sharply defined zones.
2. Among the spermatogonial cells two kinds may be distinguished, namely, the primary and the secondary.
3. The nutritive cells probably have a common origin with the spermatogonial cells. The view that the latter may be transformed from the former seems to be erroneous.
4. The number of chromosomes appearing during spermatogonial divisions is forty-eight.
5. After the last spermatogonial mitosis the chromatin material is diffused into the nuclear cavity; the leptotene threads make their appearance from this uniform ground; they are separate from the beginning.
6. In the synizesis stage the chromatin threads aggregate together in the central region of the nucleus.
7. In the synapsis stage the chromatin threads fuse in parallel fashion, or, in other words, the fusion is carried out parasynaptically.
8. Through each pachytene thread a longitudinal split is distinctly discernible and the threads show a clear bouquet-like arrangement.

9. How the tetrads are formed could not be determined. It is probable that the tetrad arises by two successive longitudinal splittings of each bivalent chromatin thread.

10. The number of chromosomes appearing in the division of the primary spermatocyte is twenty-four.

11. The chromatoid body may be found throughout all of these successive spermatogonial and spermatocyte stages, scarcely undergoing any modification; during mitoses it is situated outside the spindle.

12. After the division of the secondary spermatocyte the nucleus exhibits a reticular appearance and then, after going through a series of fairly complicated changes, it becomes universally granular in appearance and loose in consistency.

13. The centrosome wanders toward the nucleus and becomes completely enclosed within the latter.

14. A vacuole appears in the cytoplasm and enlarges to occupy by far the greater part of the cell, so that the nucleus becomes quite eccentric in situation, and finally comes into contact with the cell membrane.

15. The centrosome within the nucleus is divided into two halves, of which one representing the proximal centrosome becomes rod-shaped, while the other, representing the distal centrosome, remains unmodified.

16. Hand in hand with the changes of the centrosome, the nucleus increases its consistency and stains very heavily, and, further, appears entirely homogeneous and compact.

17. A conical prominence representing the perforatorium develops from the nucleus at the point where this is in association with the cell membrane, and the rod-shaped proximal centrosome takes the axial position in it.

18. In the spermatid are a chromatoid body and a mitochondrial mass; they do not appear to play any important part in the formation of the mature spermatozoon.

19. The mature spermatozoon is a spherical vesicular corpuscle, at one pole of which the head is attached; the head consists of two distinct parts, namely, a main part with homogeneous consistency and a subordinate part, or the perfora-

torium, represented by the conical prominence; within the head are enclosed the two centrosomes, of which the one standing in the axis of the perforatorium is rod-shaped.

20. The resemblance of the spermatozoon of *Squilla* with that of the Decapoda and particularly of the forms belonging to the Reptantia is of superficial rather than real morphological nature.

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EXPLANATION OF PLATES

All the figures excepting figure 1 were drawn at the level of table with the aid of the camera under a Zeiss 1.5-mm. apochromatic objective and a compensating eye-piece no. 8 (tube length, 160 mm.). The combination affords a magnification 2350 diameters.

ABBREVIATIONS

<i>c</i> , centrosome	<i>p</i> , perforatorium
<i>c.1</i> , proximal centrosome	<i>pr</i> , proliferating region
<i>c.2</i> , distal centrosome	<i>p.spq</i> , primary spermatogonium
<i>k</i> , chromatoid body	<i>spc</i> , spermatocyte
<i>m</i> , mitochondrial body	<i>v</i> , vesicle
<i>nt</i> , nutritive cell	

PLATE 1

EXPLANATION OF FIGURES

1 Cross-section of the testicular tube, showing the zonal arrangement of the seminal elements; there are three zones in the figure; at the upper pole is the proliferating region. $\times 200$.

2 Secondary spermatogonia; in the cytoplasm are chromatoid bodies of varying shapes and sizes.

3 Primary spermatogonium.

4 and 5 Equatorial plates of the spermatogonial mitoses, showing forty-eight chromosomes.

6 Side view of the spermatogonial metaphase.

7 A cluster of nutritive cells.

8 Preleptotene stage.

9 Leptotene stage.

10 Synizesis stage.

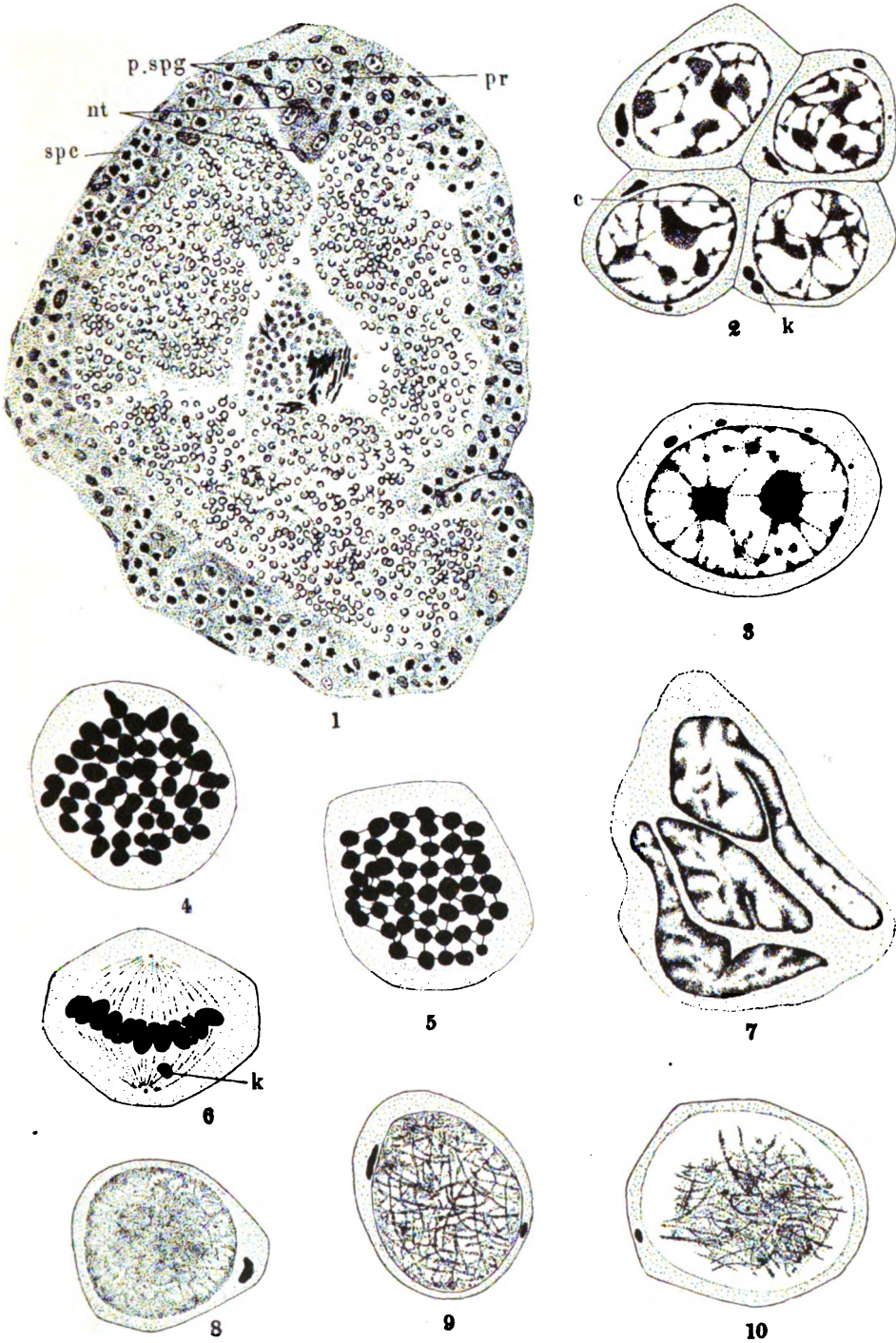


PLATE 2

EXPLANATION OF FIGURES

- 11 and 12 Synapsis stage, showing the parallel arrangement of paired threads.
- 13 Pachytene stage, showing longitudinal splittings in each thread.
- 14 Pachytene stage, showing the bouquet-like arrangement of threads.
- 15 and 16 Diakinesis stage.
- 17 Tetrads.
- 18 Tetrads transformed into rods, dumb-bells, etc.
- 19 and 20 Equatorial plates of the first maturation mitosis showing twenty-four bivalent chromosomes.
- 21 Side view of the first maturation metaphase.
- 22 to 25 Successive transformation stages of the spermatid up to just before the formation of the reticular nucleus.

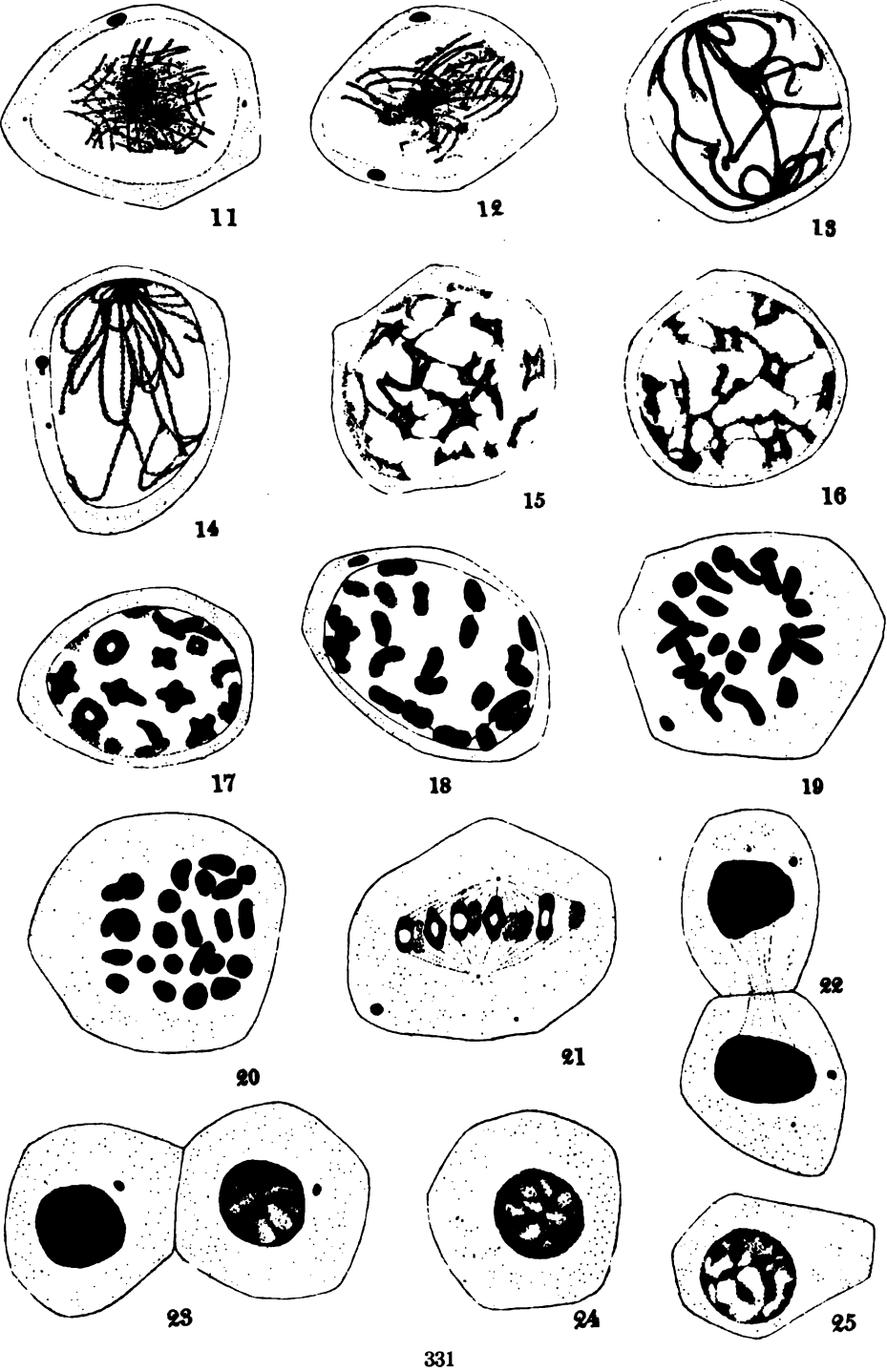
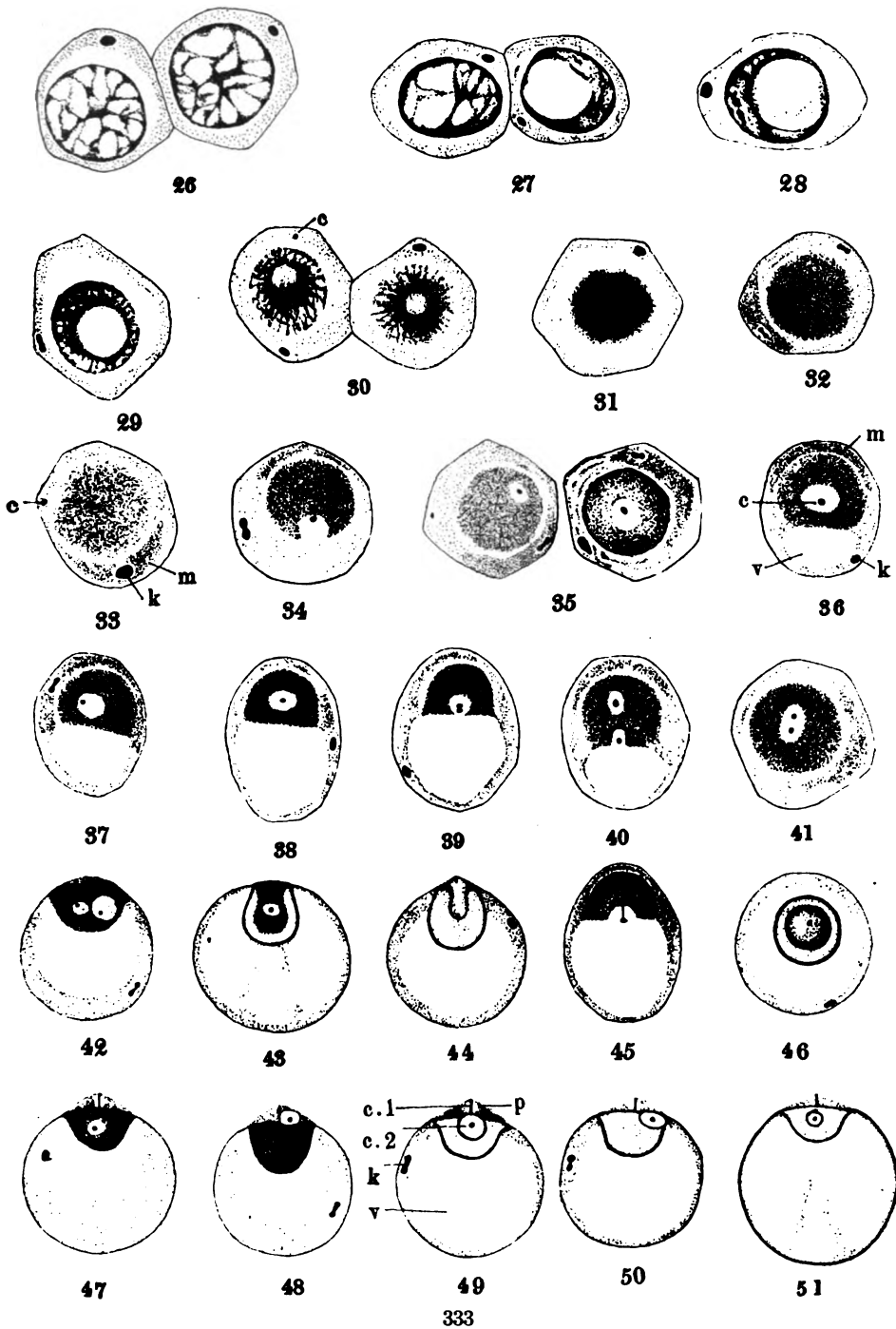


PLATE 3

EXPLANATION OF FIGURES

- 26 Spermatid with the reticular nucleus.
- 27 to 33 Successive transformation stages of the spermatid up to the formation of the nucleus with granular appearance and loose consistency.
- 34 Wandering of the centrosome into the nucleus.
- 35 Centrosome imbedded within the nucleus.
- 36 Appearance of a vacuole in the cytoplasm.
- 37 and 38 The vacuole enlarges; the nucleus diminishes its size and its staining activity increases.
- 39 Division of the centrosome.
- 40 Two daughter centrosomes lying apart from each other.
- 41 Two daughter centrosomes contained in a common clear space.
- 42 Change of the shape of the nucleus.
- 43 Consistency of the nucleus changing into homogeneous, the change advancing from the peripheral part centrad.
- 44 Change of consistency of the nucleus completed; the proximal centrosome undergoing transformation.
- 45 Proximal centrosome transformed into rod shape while associating with its fellows.
- 46 Spermatid of about the stage shown in figure 44 viewed from the top.
- 47 to 50 Nearly mature spermatozoa; the proximal centrosome lies in the axis of the perforatorium; the vacuole containing the distal centrosome lies in figure 47, within the main part of the head; in figure 48, within the perforatorium, while in figures 49 and 50 on the boundary between the two; in figures 47 and 48 the head shows a granular appearance, while in figures 49 and 50 it is perfectly homogeneous; in cytoplasm is a chromatoid body.
- 51 Mature spermatozoon.



Resumen por el autor, J. Rollin Slonaker.
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Algunos cambios morfológicos originados por adaptación en el topo.

En el presente trabajo el autor da una breve descripción de las actividades del topo, con el fin de relacionar sus cambios morfológicos con el medio ambiente. Como este animal es cavador, los ojos han degenerado hasta el extremo de perder su función como órganos visuales. Para compensar esta pérdida de la vista se desarrollan pelos sensitivos especiales en las manos y hocico. Puesto que la actividad principal del topo es cavar, su fuerza principal está concentrada en el cuarto anterior, cuyos huesos y músculos estan muy modificados y desarrollados. Los cambios mas visibles tienen lugar en el esternón, escápula, clavícula, húmero y huesos de la mano y en los músculos que mueven estos huesos. El aumento de tamaño de la región pectoral está relacionado con una reducción relativa de la pelvis, para que el topo pueda voverse en su agujero. Los orificios de salida de la pelvis han disminuido tanto a consecuencia de esta reducción que no queda espacio para el paso de los tubos digestivo y urogenital, los cuales están situados ventralmente a la sínfisis pubiana, una posición muy poco común en los mamíferos.

Translation by José F. Nonidez
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SOME MORPHOLOGICAL CHANGES FOR ADAPTATION IN THE MOLE

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FOUR PLATES (TWENTY-FOUR FIGURES)

The mole, owing to its subterranean habits, has developed some very remarkable morphological changes in some of its anatomical parts which specially adapt it to its environment. Some of these changes show marked differences from the general mammalian type.

The most prominent changes are: the degeneration of the eyes; the great increase in size of the pectoral girdle and anterior limbs; and a relative reduction in the pelvic girdle and posterior limbs, accompanied by a shifting in position of the urogenital canal and the posterior part of the alimentary tract with reference to the pelvic arch.

The various comparative anatomies and special articles which I have been able to examine have described some of the modifications as found in the European mole, but have been very meager in the discussion of the relations of these modifications.

The purpose of this paper is twofold: First, to bring together in as compact a form as possible the results of former investigators which are now scattered promiscuously and often in a more or less fragmentary manner throughout the literature and to correlate these results with the present investigation. Second, to describe these modifications in the American mole and to correlate them as far as possible with each other and with the habits and environment of this animal.

All the figures in this article were made from the American mole, *Scalops aquaticus*, of the Mississippi Valley. In the description some other species are included, especially that of the

common mole, *Scapanus latimanus*, of the central California coast region.

The absence of light has resulted in degeneration of the eyes to such a very rudimentary condition that they are doubtless able to function only in perceiving the difference between light and darkness. These structures have been previously described (Slonaker, '02). To compensate for this deficiency special touch organs have been developed in the snout and margins of the forefeet.

The manner of its subterranean locomotion necessitates very powerful fore limbs and pectoral girdle to excavate the burrow. To accomplish this a marked increase in size, accompanied by many changes in form of these parts has been brought about. This great increase in size of the pectoral girdle and fore limbs has made this region of the body very bulky. In order that the mole may turn in its burrow and go in the opposite direction, it must be able to get this bulky anterior portion by the posterior part of its body. This brings about the reduction of the pelvic girdle and posterior limbs. This reduction of the pelvis has been so great and the pelvic outlet has become so small that the urogenital and alimentary tracts cannot pass through it. These canals are therefore excluded and pass wholly ventral to the pubic symphysis. These modifications will be described in detail later on.

In order to make a definite correlation between the various modified structures in the mole and its activities and habits, it will be necessary to describe the latter in some detail.

The moles are widely distributed and are found in almost all warm and temperate regions with humid climate. Regardless of this wide distribution, there is a remarkably close resemblance in their structure. The genera of different continents show a greater difference than those of the same continent. The five genera into which True ('97) divides the moles of America show some characteristic distinctions from the European mole, *Talpa europaea*.

The European mole has been known to scientists for a long time. The earliest mention of it which I have found is in Konrad

Gesner's Thierbuch (1563). He has represented this animal in a drawing as having fairly large and conspicuous eyes, furry tail, and the toes of the front feet separated some distance. He also shows the fore feet much smaller than they should be. In his description he says that the eyes are small, but visible when the hair is parted; that the hands are large; that they are the only animals which live throughout their lives underground; that the diet consists of worms. He evidently had a fairly clear conception of some of the anatomical structures and habits of this animal.

An American mole was described and compared with *Talpa europaea* by Barrington as early as 1772. From his description, which is confined largely to the teeth, one would agree with him that it was most likely *Scalops aquaticus* which he had under observation.

The mole is almost completely covered with a soft velvety fur which can be brushed readily in any direction. The snout, the tail, a narrow band close to the feet, and the feet are not covered with fur. The legs, with the exception of the feet, are largely inclosed in the body skin and are therefore inconspicuous. The hair or fur of the mole is similar in arrangement to the wool of the sheep as described by Goette ('68).

Leydig ('59) says the general arrangement of the hairs forming the fur in mammals consists of a stiff hair surrounded by a group of fine wool hairs, all of which emerge from a common opening in the skin. The difference between the stiff hair in the center of the group and the tactile hairs is in size and nerve supply. The tactile hairs are larger and much better supplied with nerves.

. True ('97) describes the fur of the mole as very fine, velvety, slightly crenulate, and with broad, shining tips.

Wood ('10) says the fur is arranged vertical to the body, is silky, and offers little resistance to movement in any direction. It is 1 cm. long on the back. The basal portion is kinky for four-fifths the length, while the distal one-fifth is straight and bent at an angle to the other portion. This gives all the slope there is to the hair. Examined microscopically, these hairs

appear flattened. The color of the basal portion is due to alternate black and translucent bands. The apex is broader, lanceolate in shape, and contains a core of brownish-orange coloring matter.

Owen ('66) gives the following description:

. . . . the stem of the hair is filamentary, the end broad and flat, and the slender and expanded parts may alternate twice or oftener in the course of the hair, enabling the whole fur to assume any direction in which it may be stroked.

A more detailed description is given by Jackson ('15), as follows:

The hair of all American moles is fine and silky, producing a soft and velvetlike pelage. In *Scalopus*, *Scapanus*, and *Parascalops* the hairs are nearly equal in length and there is no distinct underfur. In *Condylura* some of the hairs are distinctly longer and coarser than the major portion, the latter forming an underfur, and the whole producing a pelage much less velvetlike than that of any other genus. In *Neurotrichus* the condition of the pelage is somewhat as in *Condylura*; the fur is shorter, however, and the underfur difficult to detect.

The basal pelage reveals a series of transverse vermiculations, most pronounced in *Scalopus* and *Scapanus*, least in *Neurotrichus*; in all genera these markings are more noticeable in the fur on the back, less on the ventral parts. Microscopic examination shows that these vermiculations are due to structural as well as chromatic differences. Each hair consists of normally pigmented, gray, cylindrical sections, 1 to 2 mm. long, alternated with finer flat sections, 0.2 to 0.5 mm. long and unpigmented, or with the pigment reduced to a small amount of yellow. Each one of these fine, flat sections acts as a hinge upon which the hair bends; this in part produces the velvetlike texture of the pelage and permits the hair to be rubbed either forward or backward with little friction—a distinct advantage to a subterranean mammal.

de Meijere ('94) mentions the fact that in *Talpa* the tail and the back of the hands and feet are covered with thin horny scales. Behind each of the scales on the tail there are located from four to six hairs, arranged in a fairly definite manner. He further says that in the embryo the arrangement of the hairs is quite distinct, but in the adult this arrangement is almost lost.

Reh ('95) says that the scales on the tail of *Scalops* are arranged in rings. Behind each of the scales there are about four

hairs. In *Scapanus* the scales of the tail have small tubercles. In *Talpa* the scales do not form distinct rings.

We thus have in the mole a protective covering well adapted to the environment and habits of the animal. The structure of the fur is such as to offer the least possible resistance to movement in any direction. The parts exposed most in the process of digging are either covered by scales or coarse hairs, or both, an arrangement which is well suited to the use of these parts, enabling them to be kept more free from dirt. As will be described later, some of these hairs are sense organs, and as such can serve their function best when they are isolated.

The fortress of the mole, according to Paycraft ('08), follows no fixed design, but varies according to conditions. The complicated galleries are incidental and have no reference to premeditated escape from danger. He believes the breeding fortress is built by the female and inhabited only by her. The male has a somewhat more complicated place of his own. The female begins building her fortress in autumn or early winter. The young, from two to seven in number, leave the nest in June or July at the age of about five weeks. The numerous tunnels at the nest are made partly, at least, to provide a place to put the dirt excavated in building the nest. The fortress is about 1 foot high and sometimes 3 feet in diameter. The outer fortress incloses a hollow chamber beneath which the nest is located. It consists of a ball of grass and leaves about 6 or 7 inches in diameter.

The location of the fortress or nest, according to Wood ('08), is under logs, stumps, or deep under the surface. The nest never harbors more than one adult mole (Scheffer, '13). The runways which extend out in all directions from the nest are used mainly as highways to the feeding grounds, which are reached by lateral tunnels from the main passages. Le Court ('88) says that these main burrows are larger than the others, but are not large enough for two moles to pass each other in them. He also says that different individuals may use the same passage, but they "never intrude upon each other's hunting-ground." Wood ('85) claims that deeper in the soil there is often a much larger burrow which

is sufficient to allow two individuals to pass each other. He considers this one of the main thoroughfares which lead from one feeding ground to another.

The method of making the burrow is described by Wood ('10) as follows: "The burrows of the mole are always excavated, not by bringing dirt to the surface but by pushing it aside." This is quite different from the method used by most burrowing animals.

The head is lowered and retracted—the flexibility of the neck permitting this—the fore paws are thrust forward in front of the nostrils, and by a sort of swimming motion the earth is pushed aside, the head at the same time being advanced and raised. The flexible snout is kept in continual motion probably for exploring rather than for loosening the soil, as was once thought.

Cuvier (1817) seems to be responsible for this idea, for he says that the nose is armed with a peculiar bone to assist in digging. It is now generally conceded that Cuvier was wrong in his interpretation.

The apparent ease with which the mole makes its burrows can be gathered from Herrick's description of this process ('92).

The mole may be almost said to swim through the earth, its feet not being beneath the body, but on either side, and so armed with broad spade-like claws, and so highly provided with muscles as to glide rapidly through the soft earth. During the passage through the earth, the back and shoulders wedge the earth upward, so that the course of the animal can be followed by the observer above. During its passage the highly sensitive and vibratile snout is constantly in motion, searching for such insects, worms, etc., as may come in its way. It seems hardly likely that this organ really assists materially in loosening or removing the soil, as some have thought.

This is also emphasized by Carpenter ('57):

The form of the anterior limbs, and the powerful muscles with which they are furnished, enable the animal, not merely to dig through the soil, cutting through the roots, etc., which may traverse it, but also to throw backwards with great energy the earth which has been removed at each stroke. The hind limbs are small, and the feet feeble, in comparison with the anterior; but they serve to enable the animal to run through its galleries with great rapidity.

Though the mole appears to make its burrows with comparative ease, the amount of force required must be very great. In

order to gain some idea of the force exerted by the fore legs, I wrapped a captured mole in a strong cotton cloth. With one or two efforts of its powerful paws it tore the cloth and emerged. If one attempt to tear the cloth in a similar manner with his fingers he realizes that this small animal can do easily what is almost an impossible task for man.

The depth of the tunnels depends largely on the condition of the ground. They may be as deep as 2 feet. Sometimes, in making these deep burrows through soil that is too compact to be easily pushed aside, the mole brings the dirt to the surface and deposits it in mounds.

The diameter of the runways is from 2 to 2½ inches. The main burrows are always larger than the side branches which lead to the feeding grounds. The main burrows are also permanent, and the mole repairs any damage which they may sustain.

The length of new burrows which a mole may make during a single night varies with the condition of the soil. After a rain, which renders the soil more moist and less compact, moles are more active in making new burrows. Lydekker ('93) records the formation of a new burrow about 100 yards long during a single night. He estimates that if a man were to perform an equivalent amount of work during the same time he would have to excavate a tunnel 37 miles long and sufficiently large to easily permit the passage of his body.

The rate of locomotion of the mole through its burrow has been variously stated as fast, but the distance traveled in a given time has, so far as I can find, not been estimated. The nearest approach to a definite determination of the rate which I have seen was by Le Court ('88). He stuck straws down into the burrows at intervals leaving a portion of each extending above the ground. As the mole progressed along the burrow these straws were moved and the rate could be determined by observing the straws. But instead of giving a rate which could have been so easily calculated, he concludes that "the speed of the frightened mole was equal to that of a horse at full trot." This does not seem possible when one considers the bulky fore legs which are modified for strength and not speed.

The mole is neither strictly nocturnal nor diurnal in its habits. Scheffer ('13) pressed down the runways and observed when they were repaired. He found that 135 repairs were made during the night and 116 during the day. Of a number of repairs made in the day time 109 were in the morning and 89 in the afternoon. He made more detailed observations and found the time and number of repairs made during the twenty-four hours as follows: 9 to 10 P.M., 15; 3 to 4 A.M., 16; 9 to 10 A.M., 9; 1 to 2 P.M., 11; 5 to 6 P.M., 8. From this one must infer that, while some rhythm in the activity is noted, it is not sufficiently pronounced to say that the mole has any definite time for activity and rest. The frequent periods of activity are no doubt due to the feeding habits of the animal. Carpenter ('57) speaks of the rapacious appetite of the mole which must be frequently satisfied. If its appetite is not satisfied, its hunger amounts to rage.

The purpose of the burrows is to secure food. New burrows are made when the food supply has been depleted in the old runways. The manner in which the mole feeds is described by Allen ('12). He fed a number of earthworms to a mole in captivity and noted that it used the backs of its hands to assist in the feeding process. It ate about a dozen worms before it was satisfied. I have observed that in the process of feeding the fore feet not only assist in carrying the food to the mouth, as above described, but are used also in stripping the worms. This is done by pulling the worm between the feet and especially between the claws by a backward movement of the head. This results in forcing out most of the contents of the alimentary canal leaving the worm comparatively free from earthy material. While eating the head was in almost continual motion. I infer that this was done to enable the tactile hairs of the snout to function over a wider field. The drinking process Allen describes as being similar to 'sponging out a boat.'

In order to determine the diet of the mole, Dyche ('03) examined the stomach contents of sixty-seven individuals of *Scalops aquaticus* taken during all the months of the year excepting December and February. He found that earthworms composed one-half or more of the diet, depending on the conditions of

weather and moisture. Grubs, beetles, and some vegetable matter made up the balance. He considers the vegetable matter accidental and concludes that the damage to vegetation which the mole may produce is due to its method of digging and to mice which occupy the burrows later on.

West ('10) examined the stomach contents of thirty-four moles collected from various sources and found earthworms constituted 31 per cent; adult insects, 23 per cent; insect larva, 29 per cent; vegetable matter, 13 per cent. He concludes that the mole is beneficial and the vegetable matter was eaten accidentally.

From the examination of thirty-six stomachs Wilson ('98) concludes that the mole does not intentionally take vegetable matter as food. He considers the mole beneficial, though it may accidentally do some harm. He says that the great damage to vegetation is due largely to the mouse family, Arvicolinae, which take possession of the runways.

According to Ritzema ('98), the mole stores up food for use during the cold weather. He mentions finding 300 earthworms, each of which was decapitated, in a single nest in the winter. He also mentions the finding by Stadt of a ball of earthworms in a nest in winter. Each of the worms had from three to five of the anterior segments bitten off which led him to conclude that this was intentional. The cold weather delayed regeneration and the loss of the anterior segments prevented escape. In this way the mole was assured a supply of food sufficient to tide it over periods unfavorable for securing food in the runways.

The mole is a fossorial animal spending practically the whole of its life under the ground. It is occasionally seen on the surface at rare intervals and but for a brief time. In order to adapt it to its peculiar subterranean life, a number of structural changes have taken place.

Even though no external aural appendage is present (Huxley, '90; Herrick, '92), the sense of hearing is apparently rather acute. I have found that the auricle in *Scalops aquaticus* is represented by a ring of cartilage surrounding the auditory meatus. This forms a ring-like expansion of the skin which rises slightly

above the general surface. Cuvier (1817) says a large tympanum is present. The reason for the absence of the aural appendage is apparent. It could be of no assistance in hearing and would be a hindrance to the progress of the animal through the soil.

According to Allen ('12), the sense of smell is not very keen. He bases this conclusion on the behavior of a mole which he observed in captivity. He noticed that it did not take the worms offered until the nose was almost, if not completely, in contact with them. The tactile hairs of the snout were apparently of greater use in securing the food than the sense of smell, for they were evidently stimulated.

Since the mole lives its whole life in darkness, a marked degeneration of the eye has resulted. In the adult *Scalops* this degeneration has gone so far that it can at best only distinguish between light and darkness, and it is extremely doubtful whether the eye functions at all. It is attached to the under surface of the skin a little posterior to the angle of the mouth (Herrick, '92). An eye socket has wholly disappeared.

The size of the eye has been greatly reduced, but is visible as a small dark area lying under the skin when the fur is parted over this region. The fusion of the lids has reduced the eye-cleft to a microscopical tube which meets the eyeball at such an angle that rays of light could not enter the eye along the axis of vision. All the elements of the mammalian eye are present, but they are in such a crowded condition, due to the great reduction in size of the eyeball, that it would be impossible for them to function in a normal manner (Slonaker, '02).

Sweet ('09) has studied the eyes of *Chrysochloris hottentota* and *C. asiatica* and compared them with *Talpa* and *Scalops*, and concludes that they are more degenerate than in the latter and are practically incapable of distinguishing light and darkness.

To compensate for the loss of information from the sense of sight, the sense of touch has been developed to a remarkable degree. These tactile organs are located principally on the snout and on the hands and consist of certain hairs and end-organs which are richly supplied with nerve fibers.

To gain some idea of how these organs were utilized, a mole was placed in an aquarium with a hard dry bottom. It soon became very restless, continually moving about and probing with its nose for a means of escape. The tactile hairs on its snout and the specialized papillae are apparently the chief sources of information which guide it in locomotion.

Eimer ('71) gives a good description of the touch organs in the snout of the mole. He says the whole surface is arranged in papillae, each of which is supplied with a nerve bundle whose axis cylinders have a definite arrangement. There are from one to three in the center and these are surrounded by a ring of from fifteen to twenty other axis cylinders.

Bielschowsky ('07) has reviewed Eimer's work and made further observations. He also mentions the very rich nerve supply to Eimer's organs, as he calls them, but claims that he finds as many as thirty endings to each organ. He estimates that the total number of these organs is about 150,000. The snout is thus so richly supplied with end-organs that it is a very sensitive tactile organ. The extreme tip of the snout is especially well supplied with these nerves.

Besides this specialized tactile region, the mole has also special tactile hairs on the manus. These were discovered by Merkel in 1880. More recently Kazzander ('09) has studied the tactile hairs and describes their arrangement. They form a half-circle on the palmar margin. Some of these he considers tactile in function while others are not. He found a similar arrangement of these hairs in both sexes. He thinks their function is to assist the animal in digging and in securing its food. In a later paper ('10) he describes similar hairs extending to the toes on the hind feet of both sexes. Though these hairs on the hind feet look very much like the tactile hairs on the manus, a microscopical study shows the absence of special nerves to them. He does not, therefore, consider them tactile in function. These results have been verified by Henneberg ('15).

Beddard ('16) finds that nearly all mammals possess a tuft of from one to twenty strong vibrissae, located upon the wrist, which is innervated by a branch of the radial nerve. He has

examined only two of the Insectivora (*Centetes* and *Erinaceus*), but has not found this tuft of hairs in either of these forms. The tactile hairs on the manus of the mole may possibly correspond to this tuft, but, owing to the great modifications which have taken place in this animal, do not occupy the same relative position as in other mammals, as described by Beddard.

According to Wood ('10), there is a small protuberance situated between the eye and the ear in the mole which he thinks functions as an organ of touch. This evidently corresponds to the true vibrissae found in other mammals where they function as tactile organs.

The innervation of the palmar surface of the feet is apparently not highly specialized, for Klaatsch ('88) claims that the moles show a very primitive condition in regard to the touch organs in the balls of the feet and hands. These organs function largely in the reflexes of balancing. Since the mole does not support any of its weight on its fore feet and only a little on its hind feet, this primitive condition would be expected.

From this brief résumé one must conclude that the special sense on which the mole relies most is that of touch. This is just what one would expect when the habits and environment of the animals are considered. While the senses of smell and taste are of service in the selection of food, and the sense of hearing in giving a warning of approaching danger, touch is predominant in bridging over the gap between the environment and the coordinated movements of the animal. On this sense it depends for its continued existence. The location of these special touch organs on the snout and the edge of the manus is just where one would expect in the adaptation to its environment. The massing of Eimer's organs at the tip of the snout, their gradual diminution farther back, with tactile hairs becoming more numerous, again shows adaptation. The bare snout can be kept more free from dirt for fine discrimination than if it were covered with tactile hairs. The location of the tactile hairs on the edge of the manus is the best for guiding the movements of the fore legs in digging, in feeding, and in locomotion. Furthermore, the vibrissae on the cheek are reduced in length so that they do not

protrude beyond the fur to interfere with movements, but are long enough to function as touch organs. Finally, the very degenerate condition of the eye shows a remarkable degree of adaptation, corresponding very closely with that of other animals whose entire lives are spent in darkness (Eigenmann, '09).

Even though the receptor senses and external changes show marked deviation from the mammalian type, the most noticeable changes are in certain portions of the skeleton and the muscles which control these parts. These are most conspicuous in the pectoral and pelvic regions. These modifications which are especially adapted to the environment will now be described in detail.

In order to adapt the mole to the fossorial life which it leads, the skeleton has been greatly modified. Since the animal's chief employment consists in digging, its main strength is concentrated in the fore-quarters where the bones and muscles are developed to a marvelous extent. Plate 1 shows the enormous development of the bones of the pectoral region as contrasted with that of the pelvic. The whole pectoral girdle is pushed forward close to the base of the skull, giving the animal the appearance of having no neck. This forward displacement of the girdle is due to the great development of the manubrium or presternum (*Pst*). To compensate for this the scapula (*S*) has grown backward so far that its posterior end lies in about the normal position found in most mammals.

The sterna of the moles apparently vary with different species. According to Owen ('66) and Flower ('85) in *Talpa europaea*, it consists of six or seven parts; the manubrium or presternum, the mesosternum, consisting of four (Owen) or five (Flower) sternabrae, and the xiphisternum. In the individuals of the species under observation I have found the mesosternum consisting of but three bones, the posterior two or three of *Talpa* have evidently coalesced to form one in *Scalops* and *Scapanus* (fig. 16).

The total length of the sternum is 36.4 mm., of which the presternum forms almost half. The length of the different portions is as follows: presternum 17.7 mm.; mesosternum, 12.4 mm., the first joint being 3.8 mm., the second joint, 3 mm., the third joint, 5.6 mm.; xiphisternum, 6 mm.

The presternum is not only greatly elongated, but very much broadened into a strong ventrally projecting keel (fig. 15, K), giving a larger surface for the origin of the powerful pectoral muscles. In front the presternum widens into a blunt T-like end, 3.4 mm. wide, to which is attached the very much reduced episternum (fig. 16, *Ep st*). According to Gegenbaur ('64) and Gotte ('77), the episternum forms the articular surfaces for the clavicles. The dorsal edge of the presternum is expanded laterally into wing-like projections (*w*) extending from near the anterior end to the place of attachment of the first pair of ribs, 4 mm. from the posterior end. At the place of greatest extension they have a total width of 2.9 mm. This provides increased surface for attachment of the pectoral muscles as well as increasing the strength of the presternum for lateral strain. The second pair of ribs joins the sternum at the junction of the presternum with the first mesosternal bone. The sternebrae of the mesosternum have a uniform width of 1.9 mm. The third pair of ribs joins at the junction of the first and second sternebrae; the fourth pair at the junction of the second and third sternebrae; the fifth pair joins near the middle, and the sixth pair near the posterior end of the third sternebra. The seventh and last pair of ribs, which directly joins the sternum, is attached at the junction of the last mesosternal bone with the xiphisternum. The points of attachment of the fifth and six pair of ribs doubtless indicate the places of division of the mesosternal bone in *Talpa europaea*.

The scapula is very much elongated as compared with the ordinary mammalian type. It is 25.5 mm. long and somewhat cylindrical. According to Huxley ('90), it is as long as the combined lengths of the humerus and radius. I do not find this the case in *Scalops* and *Scapanus*. Bronn states that the scapula of *Talpa* is shorter than that of *Scalops*, having a ratio of 23:25. At the posterior end it is expanded 5.7 mm. in a horizontal and 4 mm. in a vertical direction. A deep groove, bounded on either side by a sharp crest or ridge of bone, extends on the dorsal surface from the posterior end to near the middle. This greatly increases the surface for the attachment of muscles. True ('97) states that there is a prominent tubercle at the distal end of the

inferior crest or spine in *Scapanus*. This I have verified, and have demonstrated a smaller tubercle similarly located in *Scalops*. The scapula possesses a distinct acromion (fig. 11, *Ac*), from which a strong ligament extends to the outer angle of the clavicle. This ligamentous connection was described by Bell ('39). There is no articulation with the clavicle.

The proximal end of the scapula articulates directly by means of the concave *cavitas glenoidalis* with the convex *facies articularis scapulae* of the humerus. This articular surface has a horizontal dimension of 3.8 mm. and a vertical of 1.5 mm. This gives a very firm articular surface to withstand the pull of the powerful muscles of the fore leg.

The clavicle shows a great modification from the mammalian type. It is a strong cube-like bone attached to one of the T-like processes at the anterior end of the presternum. It has the following dimensions: length 4.1 mm.; anteroposterior 3.1 mm.; dorsoventral 6.3 mm. As shown in figures 3, 15, and 16, instead of the slender elongated bone in man, it has been greatly reduced in length, while its other dimensions have been much increased. These changes have been necessary to withstand the great strain (an end thrust) which is put upon it by the humerus with which it articulates. This articular surface is oval with a saddle-like depression. It measures anteroposteriorly 3 mm. and dorsoventrally 4.4 mm., thus covering practically the entire distal end of the clavicle. When we consider the shape of this surface and that of the *caput humeri* with which it articulates, we must conclude that a pivot rotation of the humerus would be very slightly if at all possible. The main movements of the humerus relative to the axis of the body are forward and backward and up and down. Intermediate movements, however, may be made.

There is a noticeable variation in the clavicle in the five genera into which the American moles are divided. These are briefly described by Jackson (15) as follows: *Scalopus*: "clavicle short and heavy, about two-thirds as broad as long, penetrated anteroposteriorly through the center by a small foramen." *Scapanus*: "clavicle short and heavy, about three-fourths as broad as long, distinctly notched on the inferior surface, not penetrated by a

foramen." *Parascalops*: "clavicle relatively longer and weaker than in *Scalopus* and *Scapanus*, length about equal to breadth, penetrated antero-posteriorly by a foramen near the inferior border." *Condylura*: "Clavicle relatively long and narrow (for *Talpidae*), length about twice the breadth, slightly concave superiorly and convex inferiorly, not penetrated by a foramen." *Neurotrichus*: "Clavicle relatively long and narrow (for *Talpidae*), length about twice the breadth; concave superiorly, inferior surface with a flat process projecting postero-laterally; not penetrated by a foramen." The clavicle thus varies from a short stout bone two-thirds as wide as long to a relatively long bone one-half as wide as long. This modification accords fairly closely with similar changes in the manus to be described later.

The proximal end of the clavicle consists of a vertical groove 6 mm. long by 1.5 mm. broad which fits closely to the elongated convex surface of the T-like process of the presternum. This constitutes a simple hinge-joint and allows a slight movement in a forward and backward direction. Parsons ('01) claims that in the giant golden mole (*Chrysochloris trevelyani*) there is no synovial cavity between the presternum and the clavicle, but that the joint is nevertheless very flexible.

According to Parker and Haswell ('10), the clavicle in the mole represents a precoracoid as well as a clavicle. They base this statement on the method of development, claiming that the posterior part is developed from a mass of cartilage, while the anterior part, which early becomes attached to the cartilage mass and which represents the clavicle, is formed, as usual, in membrane.

The humerus of the mole would scarcely be recognized as such if isolated from its connections (figs. 1, 2, 3, 8, 9, 10). These figures show that it is extremely irregular and that it has been very greatly changed from the humerus of typical mammals. The broad, thick, and short mass of bone gives great strength, while the great irregularity increases the surface for the attachment of the powerful muscles used in digging. It is 15 mm. long, 11.9 mm. broad, and 5.4 mm. thick. The large indentation on the anterior edge, partly inclosed by the epicondylus lateralis

(fig. 8, *el*) on the distal side and the tuberculum minus (*tm*) on the proximal side, is for the passage of the flexor muscles of the forearm. These muscles have their origins in a cavity of the humerus just under the articulation for the scapula (*f*). The origins of the extensor muscles of the forearm is at the proximal and posterior margin of the humerus just posterior to the facies articularis scapulae (*f*).

There are four articular surfaces on the humerus. A proximal one, caput humeri (figs. 8, 9, 10, *ch*) which articulates with the clavicle, is somewhat oval in outline, and has almost the uniform convexity of a cylinder. It is 6.8 mm. in a horizontal and 4.5 mm. in a vertical direction. This surface being so much greater than that of the clavicle (30.6 sq.mm. vs. 13.2 sq.mm.) with which it articulates rather indicates that there may possibly be a sliding movement in an anteroposterior direction at this joint.

A second articular surface, facies articularis scapulae, (*f*), is situated on an eminence on the dorsal side near the proximal end, a short distance posterior to the caput humeri. It is almost almond-shaped and measures 3.8 mm. in a lateral direction and 1.5 mm. in a direction parallel with the axis of the body. As its name indicates, it is the articular surface for the scapula.

The third and fourth articular surfaces are located at the distal end of the humerus. They are the trochlea humeri (*tr*), the articular surface for the ulna, and the capitulum humeri (*c*), the articular surface for the radius. The trochlea humeri is shaped like a slightly bent cylinder whose length is 2.7 mm. and diameter 1.5 mm. There is a depression, the fossa coronoidea (fig. 10, *fc*) on the ventral side just proximal to the articular surface. It provides room for the coronoid process of the ulna in extreme flexion. A depression, the fossa olecrani (*fo*), similarly situated on the dorsal surface, furnishes room for the process of the olecranon in extreme extension of the forearm.

The bones of the forearm also show some marked modifications. They are both very strongly formed and reinforced. They are relatively short and thick compared with the general mammalian type. The radius, instead of the long cylindrical

shape found in man, is flattened (figs. 13 and 14). A cross-section of the shaft does not therefore approach that of a circle, but an ellipse, whose dimensions are 2.8 mm. by 1.5 mm. The greater diameter lies in the plane of movement of the forearm, thus providing increased strength in the required plane. The length of the radius is 13 mm. We thus have a ratio of the average diameter of the shaft to the length of the bone of approximately 1:6. In man the ratio is about 1:17 and in the dog 1:15. This will give a fair idea of the comparative strength of this bone. Another modification from the human is seen in the proximal articular surface, the fovea capituli radii. Instead of being a shallow socket, which allows rotation of this bone on its axis, it is a deep spherical depression which is well extended proximally on the extensor side by a strong process. This alone would make rotation of the forearm difficult. The fovea capituli radii articulates with the capitulum humeri described above. The distal end of the radius is widened in the same plane as that of the manus to 6.2 mm. This forms a strong hinge-joint which would permit little lateral movement of the hand. It articulates at the wrist with the os naviculare manus, the os lunatum, and the radial sesamoid.

The ulna shows a still greater modification than the radius. It is 18 mm. long and is extended well beyond the elbow-joint by the proximal elongation of the olecranon. The length from the distal end to the center of articulation at the elbow is 11 mm. From this articulation to the tip of the olecranon is 7 mm. Since the extensor muscles are attached to the greatly expanded end of the olecranon, we thus have a strong lever, the ratio of whose arms is as 11:7, or about 1.4:1. In the dog this ratio is approximately 7:1 and in man 14:1. This modification results in great increase in strength at a loss of speed. In order to withstand the breaking strain at the elbow-joint, the ulna is greatly strengthened or reinforced on the flexor side by a broad keel-like outgrowth, approximately 10 mm. in length and 3.5 mm. wide at its greatest width. This is broadest at the joint and gradually grows less in either direction (fig. 13). The incisura semilunaris (*is*) which articulates with the trochlea humeri (*tr*)

at the elbow, is approximately that of a hollow cylinder. Its surface is greatly increased on the distal side by the processus coronoideus and on the proximal side by a well-marked process from the olecranon. This articular surface lacks only about 90° of forming a complete hollow cylinder. As may readily be seen, this permits forceful movements of the humerus with the forearm in any degree of flexion or extension, without probability of dislocation at the elbow. Some 3 or 4 mm. from the distal end the ulna approaches most nearly a cylindrical shape. From this point distally it rapidly widens to 5 mm. at the end in the same plane as the distal extremity of the radius. This wide expansion articulates with the os triquetrum and os pisiforme. Thus at the wrist both the bones of the forearm are flattened in the same plane as that of the manus, which is also the plane of extension and flexion of the forearm. The total width of these two articulations at the wrist is 9.4 mm. This is thus reduced to a simple hinge-joint. The proximal joint is, in my opinion, also a hinge-joint. The combined width of the ulna and radial articulations at the elbow is 5.5 mm. Since both the proximal and distal joints of the forearm are hinge-joints, it is obviously impossible for these species to execute a rotary movement of the manus similar to that in man. In the giant golden mole (*Chrysochloris trevelyani*) Parsons ('01) claims that the hand can be rotated one-fourth of a circle. He states that this is possible because the proximal projection of the radius at the elbow is absent. It seems to me that the articulation of the ulna and radius at the wrist would also have to be different from that of *Scalops* and *Scapanus* to make this movement possible.

There are eight carpal bones, an os centrale being present. These are indicated in figure 13. Besides these carpal bones, there are two sesamoid bones, the os pisiforme (*p*) and the os falciforme (*Rs*). The os pisiforme is attached to the distal end of the ulna at the edge of the wrist on the exterior surface. It has a hook-like projection extending toward the palmar surface. This doubtless assists in confining the tendons of the digits to the wrist region. The os falciforme or radial sesamoid is a long, narrow, sickle-shaped bone measuring 11 mm. long, 1.2 mm. thick,

and 2 mm. wide. Its proximal end is attached to the pollex or flexor side of the distal end of the radius. From its attachment it curves outward and in the direction of the palm and along the side of it to end on the flexor side of the manus near the level of the proximal end of the second phalanx of the pollex. It thus not only adds to the width of the manus, but also serves to add firmness to this margin of the hand.

The metacarpal bones and the phalanges are of the usual number, but very much reduced in length. The terminal phalanges are bifid at their distal extremity. This has been described by other investigators in other species of moles and also in the scaly anteater, *Manis*, by Humphry ('70). Mivart ('71) says that these bifurcations are less pronounced in *Scalops aquaticus* than in *Talpa*. In figure 13 the terminal phalanx of the third digit is shown in outline as seen through the intact nail.

The manus in *Scalops* is thus broad and spade-like in shape and armed with strong flat nails which practically inclose the terminal phalanges. The palm is much broader than long. It is 17 mm. wide and 11.3 mm. long. The toes, being fully webbed, make is a very effective organ for digging. In *Scapanus* and *Parascalops* the palms are as long as broad and the toes are not webbed, but provided with broad flat claws. In *Condylura* the palms are as long as broad, the toes not webbed and relatively long and armed with rather long and somewhat narrowed claws. In *Neurotrichus* the manus is less hand-like, the palms are longer than broad and the toes are relatively long and not webbed. It is not such a powerful organ for digging as in the other genera. Likewise, the other bones of the arm are not so strongly built as in those forms with the strong spade-like manus.

The manus at rest is so placed that the palmar surface is directed mainly outward, but also slightly upward and backward. This brings the pollex edge, which is reinforced by the strong os falciforme, on the ventral edge. This edge is subjected to greater strain in the process of digging than the opposite edge, and adaptation is shown in its reinforcement. Since there can be little if any rotation of the hand, the movement at the wrist consists probably wholly of flexion and extension.

The former, in the act of digging, moves the dirt upward and backward. The flexion of the digits also assists this process as this results in making the manus more scoop-like. The movement of the forearm is such as to carry the manus nearly edge-wise in almost any direction depending on the position of the humerus. The combined movements of extension of all these parts result in thrusting the manus forward, palm out, until the

TABLE 1

Showing the number of vertebrae in the different groups of moles as described by various authors

GENERA AND SPECIES	AUTHOR	CERVICAL	THORACIC	LUMBAR	SACRAL	CAUDAL	TOTAL
<i>Talpa europaea</i>	Flower, '85	7	13	6	5	11	42
<i>Talpa europaea</i>	Bell, '39	7	13	6	6	11	43
<i>Talpa europaea</i>	Cuvier, '17	7	13	6	6	11	43
<i>Talpa caeca</i>	Cuvier, '17	7	14	5	5	11	42
<i>Talpa caeca</i>	Bell, '39	7	14	5	5	10	41
<i>Talpa</i> (species not given).....	Mivart, '67	7	13	5			
<i>Talpa</i> (species not given).....	de Blairville, '21		14	6	4-5		
<i>Talpa</i> (species not given).....	Owen, '66		14	5	5		
<i>Scalops</i> (mole shrew).....	Cuvier, '17	7	12	7	6	10	42
<i>Scalops</i> (species not given).....	Bell, '39	7	12	7	6	10	42
<i>Scalops</i> (species not given).....	True, '97	7	14	5	6	11	43
<i>Scalops aquaticus</i>	Mivart, '67		14	5	5	11	
<i>Scalops aquaticus</i>	Slonaker	7	14	5	6	11	43
<i>Scapanus californicus</i>	True, '97	7	14	5	6	13-14	45-46
<i>Scapanus californicus</i> (True)....	Slonaker	7	14	5	6	14	46
<i>Scapanus latimanus</i>							
<i>Condylura cristata</i>	Cuvier, '17	7	13	6	5	17	48
<i>Condylura cristata</i>	True, '97	7	13	6	5	19	50

claws project slightly beyond the snout. The combined flexion brings about the reverse movement and results in forcing backward and upward a scoop of dirt coupled with a forward movement of the animal. The whole process resembles very closely the movements in man when swimming.

The number of vertebrae in the moles varies with the species. In fact, different investigators have not always agreed on the same number for a given species. This is exemplified in table 1.

It will be seen that the cervical vertebrae are constant and conform to the mammalian type and that the other groups vary. The discrepancies which are noted for the same genus may be due to the fact that the different authors may not have had the same species under observation or that a variation occurs; more likely, the first supposition obtains.

The transverse processes of the vertebrae of the species under observation are relatively short. This permits a greater flexibility of the spinal column, which makes it possible for the mole to turn more easily in its burrow and go in the reverse direction.

Owen ('66) describes 'antigenous hypophysial ossicles' interposed beneath the interspaces of the bodies of the lumbar vertebrae. This is verified by True ('97). I find six or seven such ossicles in *Scalops aquaticus* and eight in *Scapanus latimanus* (fig. 3 and fig. 6, Os). The posterior one is the largest and is located at the junction of the posterior lumbar with the first sacral. The series gradually diminishes in size until the most anterior element is barely visible. These species also have ossicles situated on the ventral side at the interspaces of the caudal vertebrae. In younger animals they consist of a pair of almond-shaped bones arranged parallel with each other and with the axis of the tail. In old animals these are usually fused by median outgrowths and form a single H-shaped ossicle. The most anterior are the largest and occur beneath the intervertebral space of the first and second caudal vertebrae. They gradually diminish in size posteriorly until they disappear. *Scalops* has five such pairs of ossicles and *Scapanus* eight. Similar ossicles are found in other tailed mammals.

I am at a loss to determine what the function of these ossicles may be. The position and shape of those in the lumbar region suggest that they may act as a sort of pivot or fulcrum to increase the possible lateral movement. In the caudal region there is nothing which would indicate this possible function. The fact that tendons seem to be attached to them indicates that they function in movement of the tail.

The cervical and thoracic vertebrae are without spinous processes, permitting greater flexibility of the animal. The lumbar

vertebrae are almost uniform in shape except that the fourth has a median process extending anteroventrally from the centrum. In some cases a similar projection occurs on the fifth lumbar, but when present it is very small. I have not determined the function of this process. It is completely covered by the psoas magnus muscle and may serve to increase the surface for its attachment. The sacral vertebrae are so closely fused in the adult that they form a continuous bone to which the pelvic arch has become ankylosed for almost its entire length.

According to other investigators, the number of ribs varies from twelve to fourteen in different species. In the two American species under observation there are fourteen pairs. They are very slender and pliable, thus permitting great flexibility of the animal. This pliability is due not only to their small diameter, but also to the attachment to the vertebrae and to the greater ratio or proportion of cartilaginous portion to bony portion. The attachment at the proximal end is such as to allow relatively free movement and the large proportion of cartilage permits easy bending. The average ratio of the bony to the cartilaginous portion in *Scalops* is 1.4 to 1. In man it is approximately 5.43 to 1.

The ribs are so fragile that fracture frequently occurs during life. Evidence of such a break is seen on the sixth rib in figure 1. All the ribs are ribbon-like and measure from 1.2 to 2 mm. wide and 0.4 to 0.5 mm. thick. Their length is quite variable as seen in table 2.

The first rib is 10.8 mm. long and the seventh or longest measures 44.3, an increase of over four times. These results are at great variance to the statement of Bell ('39) who says, "The ribs in the mole and its congeners are nearly all of the same length, giving the peculiar cylindrical form to the body which characterises these animals, and which is so essential to their habits." Plate 1 shows that the thorax has the typical cone-shape characteristic of mammals. The transverse diameter of this cone at the level of the first rib is 7 mm.; at the level of the ninth or tenth rib it reaches its greatest dimension of 34

mm. The above author evidently failed to recognize that the "cylindrical form of the body" was due to most of the bones of the fore limbs being enclosed in the body integument rather than to the length of the ribs.

The pelvic girdle also shows marked modifications as compared with the mammalian type. As shown above, the pectoral girdle and the bones of the fore limbs are greatly enlarged and strengthened. In the pelvic girdle the reverse condition obtains, resulting in a great reduction in the size of the pelvis. This is

TABLE 2

Showing the lengths in mm. of the ribs, together with their bony and cartilaginous portions of Scalops aquaticus

NUMBER OF RIB	BONY PART	CARTILAGINOUS PART	TOTAL LENGTH
1	7.0	3.8	10.8
2	8.0	7.7	15.7
3	11.3	9.0	20.3
4	17.0	11.5	28.5
5	18.5	12.0	30.5
6	23.7	14.5	38.2
7	26.0	18.3	44.3
8	26.0	17.5	43.5
9	25.0	16.5	41.0
10	24.5	16.0	40.5
11	22.0	15.5	37.5
12	18.0	14.5	32.5
13	16.0	14.0	30.0
14	12.0	12.0	24.0

especially noticeable in the diameter. As previously stated, this reduction in size is necessary to permit the bulky anterior portion to pass the posterior part when the mole turns in its burrow. Accompanying this reduction in diameter the pelvis has taken a position almost parallel to the vertebral column. According to Taylor ('14), this arrangement would tend to increase the flexibility of the animal, for he says: "In aquatic animals increased flexibility of the vertebral column is associated with a pelvis having a position more nearly parallel to the vertebral column than in land forms. The pelvis is also more extended

posteriorly, and has a looser connection with the sacrum and weaker pubic symphysis." This possibility may be offset, as shown farther on, by the fact that there is a very firm bony connection between the pelvis and the sacrum in moles.

All the bones of the normal pelvis are present. These bones have been so coössified that the lines of union are practically obliterated. An attempt has been made to indicate their boundaries as nearly as they could be determined in figures 4, 5, and 6.

Wiedersheim says that the 'pars acetabularis' in the mole (*Talpa*) is strongly developed and "shuts the ilium as well as the pubis out of the acetabulum: in by far the greater number of mammals the pubis only is thus excluded."

Figures 2 to 6 show that the pelvis has been greatly narrowed and that the sacral vertebrae have completely coalesced with the ilium and ischium; the space between the sacrum and pelvis is thus entirely bridged by bone with exception of two pairs of foramina (figs. 2 and 5). This bridge is 18 mm. long in an antero-posterior direction and constitutes about 64 per cent of the entire length of the pelvis. The pelvis of the species of *Scalops* under consideration has the following dimensions: Extreme length, from anterior end of ilium to posterior tip of ischium, 27.8 mm. Width, at posterior tips of ischia, 9.5 mm.; at anterior ends of ilia, 9.8 mm.; from socket to socket, 6 mm. The dorsoventral diameter in the region of the acetabulum from the dorsal margin of the ischium to the pubic bones is 4.5 mm. From the dorsal edge of the spinal crest through the acetabulum to the pubic bones is 7 mm. The transverse diameter of the pelvic outlet at the posterior margin of the pubic symphysis is 3 mm. (fig. 7); at the anterior margin it is 2 mm. The dorsoventral dimensions at these same regions are 1.5 mm. and 1.25 mm., respectively. At the anterior margin of the pubic symphysis a sharp median wedge-like projection extends ventrally from the centrum of the sacrum to within 1 mm. of the pubic symphysis. The pelvic outlet in this region is thus almost divided into two very small passages, each about 1 mm. in diameter. The change in the location of the alimentary and urogenital tracts which result from this condition will be discussed later.

To give a better idea of the comparative diameter of the body in the pectoral and pelvic regions, the following measurements of the skeleton are given. Width from the outer margin of one humerus to a similar point on the other 32 mm. Width from outer surface of the head of the femur to a similar point on opposite side 18 mm. This constitutes the greatest width of the skeleton in the region of the pelvis. Width from tip to tip of the claws of the hands about 72 mm. Outside measurement from presternum to top of skull 33 mm. Dimensions at greatest expansion of ribs (about the tenth rib) width 34 mm.; dorsoventral diameter 22 mm. By comparing these figures it is readily seen that the pelvis is only one-fourth of the greatest width in the pectoral region.

The greatest variation found in the pelvis in the different genera of the Talpidae is in the region of the pubic symphysis, which varies from a well-formed symphysis to a relatively wide gap between the pubic bones.

In *Scalops* the pubes form a symphysis which extends for a distance of 3.8 mm. parallel with the sacrum. In *Scapanus* Jackson ('13) says, "bones of opposite side scarcely touching under acetabula." In the specimen of *Scapanus* examined I find that the pubes meet for a distance of 0.5 mm. This union is due to slight projections on the medial side of the pubes and occupies a position similar to the anterior margin of the symphysis in *Scalops*. It also lies in the vertical plane passing through the anterior margins of the acetabula.

In *Parascalops* and *Condylura* Jackson ('15) says that the bones of the opposite side do not touch under the acetabula and that in *Neurotrichus* they are separated by a space of about 2 mm. True ('97) also makes a similar statement regarding these genera. The European mole, *Talpa europaea*, has been most widely studied. All investigators agree that in this species there is a rather wide gap between the pubic bones. In fact, this knowledge is so common that the mole in general is spoken of as having no pubic symphysis. This is exemplified by Leche ('80) who proposed to classify the Insectivora into three groups as follows: *a.* with long pubic symphysis; *b.* with short pubic

symphysis, and, *c.* with no pubic symphysis. Under this last group he placed the Talpidae and considered it the most primitive group.

Many authors, when stating that there is no pubic symphysis in the mole, have under consideration the species *Talpa europaea*. But the general statement so often found that a pubic symphysis does not exist in the Talpidae will have to be modified.

Another modification is seen in the dorsal spines of the sacrum. These have coalesced to form a median dorsal crest 20 mm. long and from 2 to 3 mm. high extending almost the entire length of the sacrum. This crest and the long bridges between the sacrum and the pelvic bones provide sufficient strength to compensate for the weak or complete absence of the pubic symphysis.

The hind legs of the mole are more frail than is usual in a mammal of this size. In the femur, the tibia and fibula (pl. 4) there is no great modification in the form of these bones. The tibia and fibula are united for more than half their length from the distal end. The proximal ends of each of these bones have a decided hook-like process on the lateral side, which extends outward and downward, approaching each other somewhat toward their tips. The foot is plantigrade. It is relatively long and slender, measuring from 22 to 25 mm. from heel to tip of claws. The claws are slender. The whole foot suggests weakness.

Owing to the great reduction in the pelvic outlet, a most remarkable change has occurred in the relation of the urogenital and alimentary tracts to the pelvic arch. The outlet is so small that it is impossible for these tracts to pass through the arch as in most mammals and still function. The result is that they are wholly excluded and lie ventral to the pubic symphysis.

That this relationship in the mole was known to naturalists long ago is evidenced by the following quotation from Wood's ('59) article on the pelvis in Todd's Cyclopaedia of Anatomy and Physiology. He says:

The Mole (*Talpa*) and the Shrews (*Sorex*) etc. are remarkable for a very narrow sacrum, composed, according to Blainville, of four vertebrae, but, according to Cuvier, of seven in the Mole and three in the

Shrews. In the Mole the ilia are solidly ankylosed to nearly the whole length of the sacrum. In the Shrews the two first only of the sacral pieces are united with the ilia. The spines in both are coalesced into a prominent sacral crest. Caudal pieces numerous.

The ilia are cylindrical, much approximated, and parallel to the spinal column. The ischia are much elongated, and elevated posteriorly above the sacral vertebrae. The pubes are very short and slender, and though they unite with the short ischial rami to inclose a small obturator foramen, do not meet in a symphysis, but present an anterior interval, said to be wider in the female than the male, and causing the whole pelvis to assume very much a bird-like appearance. The pelvic cavity and outlets are so straight that the sexual and urinary organs and rectum pass altogether in front of it.

Later Leche ('80), describing the embryo mole of 35 to 41 mm. body length, says that a cartilaginous band connects the posterior angles of the pubic bones, completely closing off the pelvis on the ventral side. Through this enclosed space the urogenital and alimentary tracts pass in a normal manner. He also states that the pubic bones in the embryo of *Talpa* are wider apart than in the adult. The same statement is found, word for word, in volume 1 of Bronn's *Klassen und Ordnungen* (p. 581).

Ferner sind die beim erwachsenen Thiere einander so nahe gelegenen vordersten Schambeintheile (in der Acetabulargegend) beim Embryo weit von einander entfernt. In diesem geräumigen, in der Acetabular-region offenen, im hintersten Theile ventralwärts geschlossenen Becken liegen in ganz normaler Weise Urogenitalsystem und Mastdarm und gehen, ventralwärts vom obengenannten Knorpelbande umschlossen, durch das Becken. Auch bei Embryonen von Soriciden fand ich ein Schamfugenligament.

Leche further remarks in a footnote that in the adult mole, as first described by Jacobs, 1816 (no reference given), the urogenital and alimentary tracts run outside of the pelvis and on the ventral side. This is the earliest reference I have found referring to this condition.

The abdominal cavity extends well back posterior to the pelvis, carrying with it the openings of the urogenital and alimentary tracts. This fact was known to Bell ('39) for, in discussing the mole, he says:

Another peculiarity in this animal is that the abdominal cavity, being extended greatly beyond the pelvis, the vagina, the rectum, and the urinary passage terminate considerably further back than in other animals. The opening of the rectum is opposite to the articulation of the fourth with the fifth caudal vertebrae.

This is a unique condition which I believe is restricted to the Talpidae. It gives to the posterior part of the animal a conical appearance and makes the body appear longer. This apparent increase, however, in no way interferes with the flexibility of the animal.

SUMMARY

The fossorial habit of the mole has resulted in practically the loss of the sense of sight. To compensate for this, special tactile organs have been developed.

To accomplish the digging, the sternum, pectoral girdle, and fore limbs have been greatly enlarged and modified, thus increasing the size of this part of the body.

To allow the increased size of the anterior part of the body to pass the posterior part when the animal turns in its burrow; the pelvic girdle has been very much reduced in diameter.

The reduction in the size of the pelvis has so narrowed the pelvic outlet, that it is impossible for the urogenital and alimentary tracts to pass through it and still function. This necessitated their passage outside of the bony arch—a condition very unusual in mammals.

All these remarkable modifications from the mammalian type specially fit the mole for the peculiar environment in which it lives.

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PLATE 1

EXPLANATION OF FIGURES

- 1 Photograph of the lateral view of the adult skeleton.
- 2 Photograph of the dorsal view of the adult skeleton.
- 3 Photograph of the ventral view of the adult skeleton.

ABBREVIATIONS

C, ridge-like projection from the ankylosed sacral vertebrae; *Cc*, coracoclavicle; *F*, femur; *Fb*, fibula; *H*, humerus; *Il*, ilium; *Is*, ischium; *P*, pubes; *Pst*, manubrium (presterneum); *Rs*, radial sesamoid; *S*, scapula; *T*, tibia; *U-R*, ulna and radius.

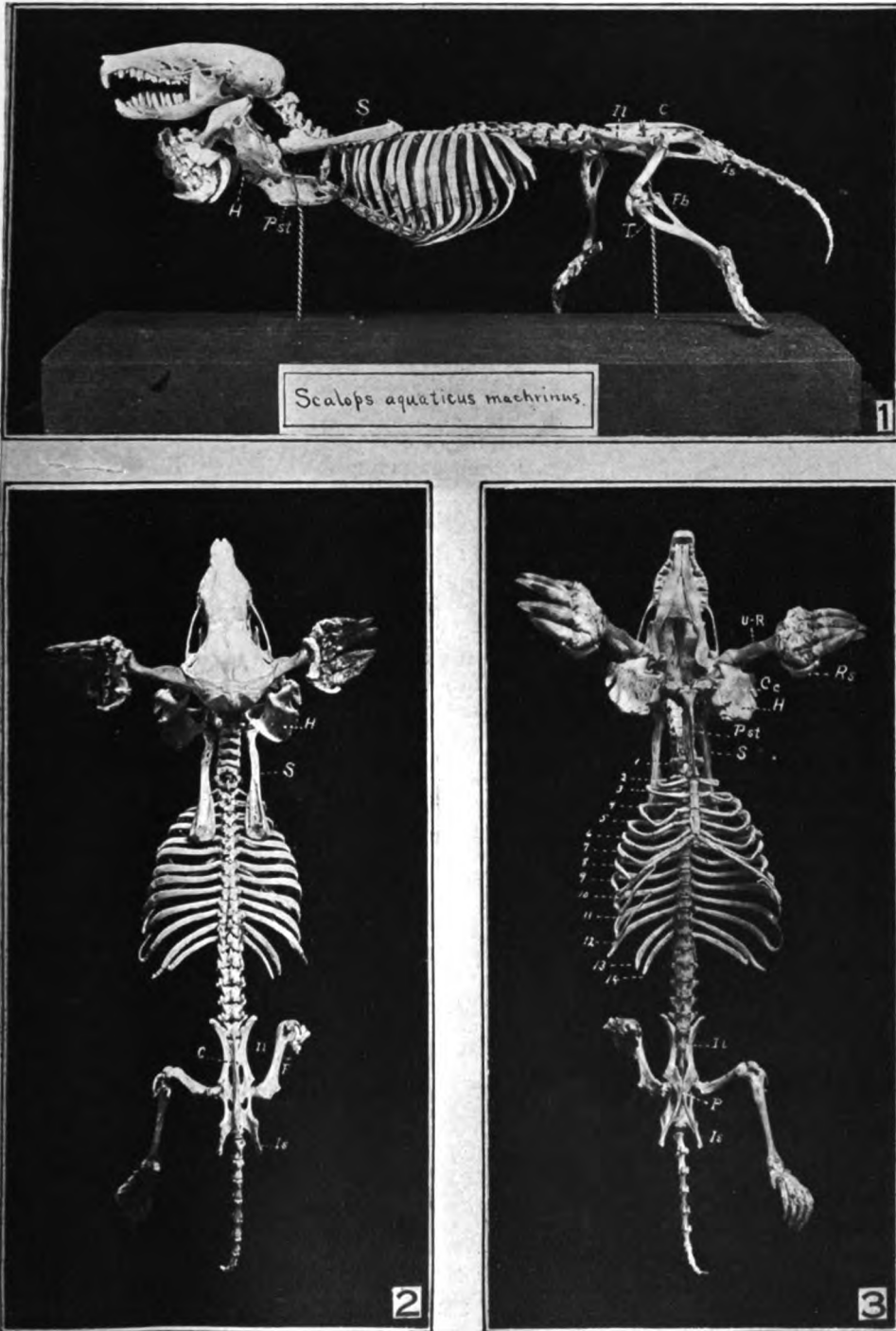


PLATE 2

EXPLANATION OF FIGURES

- 4 Photograph of the lateral view of the pelvic girdle after removal of the femur. Enlarged.
- 5 Photograph of the dorsal view of the pelvic girdle. Owing to the dense printing, the outlines of the posterior extensions of the ischia are outlined in white. Enlarged.
- 6 Photograph of the ventral view of the pelvic girdle. Enlarged.
- 7 Photograph of the pelvic girdle from the caudal end after removal of the caudal vertebrae. The greatly reduced pelvic outlet (*Po*) is shown. Enlarged.

ABBREVIATIONS

A, acetabulum; *C*, crest-like ridge extending dorsally from the ankylosed sacral vertebrae; *Il*, ilium; *Is*, ischium; *H*, hypapophyses; *Of*, obturator foramen; *P*, pubes; *PA*, pars acetabularis; *Po*, pelvic outlet. The black lines indicate as nearly as could be determined the places of junction of the various bones.

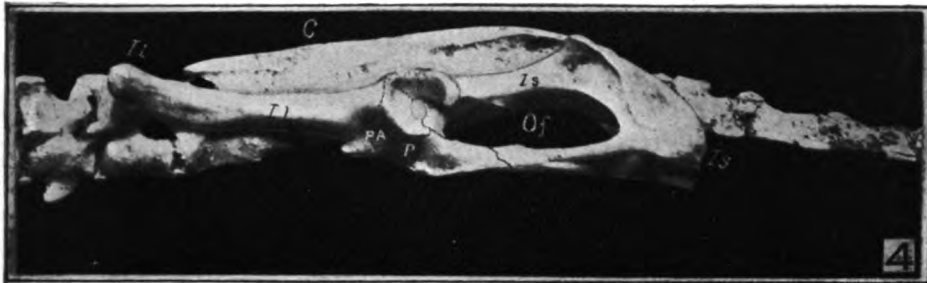


PLATE 3

EXPLANATION OF FIGURES

8 Approximately dorsal view of right humerus, showing its broad lateral expansion and the surface for articulation with the coracoclavicle, *Ch*; with the scapula, *f*; with the radius, *c*, and with the ulna, *tr*.

9 Approximately anteromedian view of right humerus showing its relationship to the forearm (fig. 13), and the scapula (fig. 11). The dotted line from the capitulum humeri, *c*, to the fovea capituli radii and incisura semilunaris of figure 13 indicates the point of union at the elbow-joint. The dotted line from the facies articularis scapulae, *f*, to the cavitas glenoidalis, *Cg* of figure 11, indicates the union of the humerus with the scapula. Figures 9, 11, and 13 are each drawn from practically the same point of view.

10 An approximately ventral view of the right humerus. The two dotted lines from the capitulum humeri, *c*, and the trochlea humeri, *tr*, to the fovea capituli radii, *fr*, and the incisura semilunaris, *is*, of figure 14 indicate the points of junction of these bones at the elbow-joint. The proximal end of the humerus would have to be lifted up until the figure made almost right angles with the paper and the figure 14 to give the normal relative position of these bones.

11 Anterodorsal view of the scapula.

12 Lateral view of the scapula.

13 Anteromedian view of the ulna, radius, and manus.

14 Ventral view of the ulna, radius, and manus.

ABBREVIATIONS

Ac, acromion; *c*, capitulum humeri; *Cg*, cavitas glenoidalis (acetabulum humeri); *Ch*, caput humeri; *ec*, epicondylus medialis; *el*, epicondylus lateralis; *f*, facies articularis scapulae; *fc*, fossa coronoidea; *Fi*, fossa infraspinata; *fo*, fossa olecrani; *fr*, fovea capituli radii; *Fs*, fossa supraspinata; *is*, incisura semilunaris; *O*, olecranon; *pc*, processus coronoideus; *p*, pisiforme; *R*, radius; *Rs*, radial sesamoid; *Ss*, spina scapulae; *t*, for attachment of triceps muscle; *td*, tuberositas deltoidea; *tm*, tuberculum minus; *tm'*, tuberculum majus; *tr*, trochlea humeri; *U*, ulna; *V*, ventral surface; *1*, *2*, and *3*, first, second, and third phalanx, the last one covered by the claw; *I*, *II*, *III*, *IV*, and *V*, ossa metacarpalia; *l*, os naviculare manus (scaphoid); *2*, os lunatum (lunar); *3*, os triquetrum (cuneiform); *4*, os multangulum majus (trapezium); *5*, os multangulum minus (trapezoid); *6*, os capitatum (magnum); *7*, os hamatum (unciform); *8*, os centrale. All figures have the same magnification.

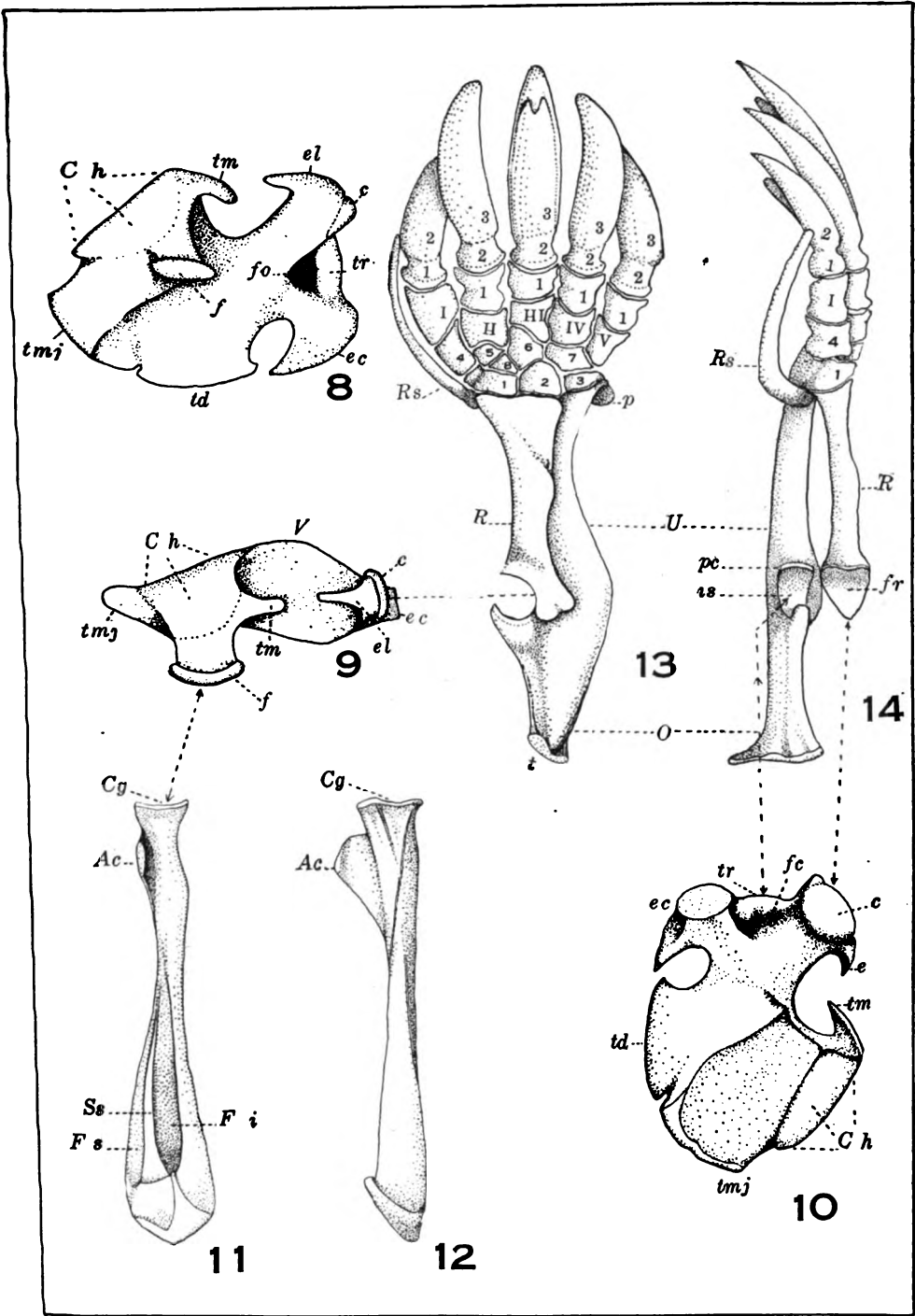


PLATE 4

EXPLANATION OF FIGURES

15 Enlarged side view of the sternum, showing the attachment of the first seven ribs (*1* to *7*), the coracoclavicle (*C cl*), and the relative position of the vertebral column (*Vt*) and the skull (*Sk*). The decided keel-like extension (*k*) of the manubrium (presternum) and its great extension forward is noted.

16 Enlarged ventral view of the sternum, showing the attachment of the first seven pairs of ribs (*1* to *7*) and the coracoclavicles. The widened anterior end and the wing-like projections (*w*) of the manubrium are noticeable.

17 Enlarged view of the femur from anterior aspect.

18 Enlarged view of the femur from the posterior aspect.

19 Enlarged view of the femur from the lateral aspect.

20 Enlarged view of the tibia (*T*) and the fibula (*F*) from in front.

21 Enlarged view of the tibia (*T*) and the fibula (*F*) from the posterior aspect.

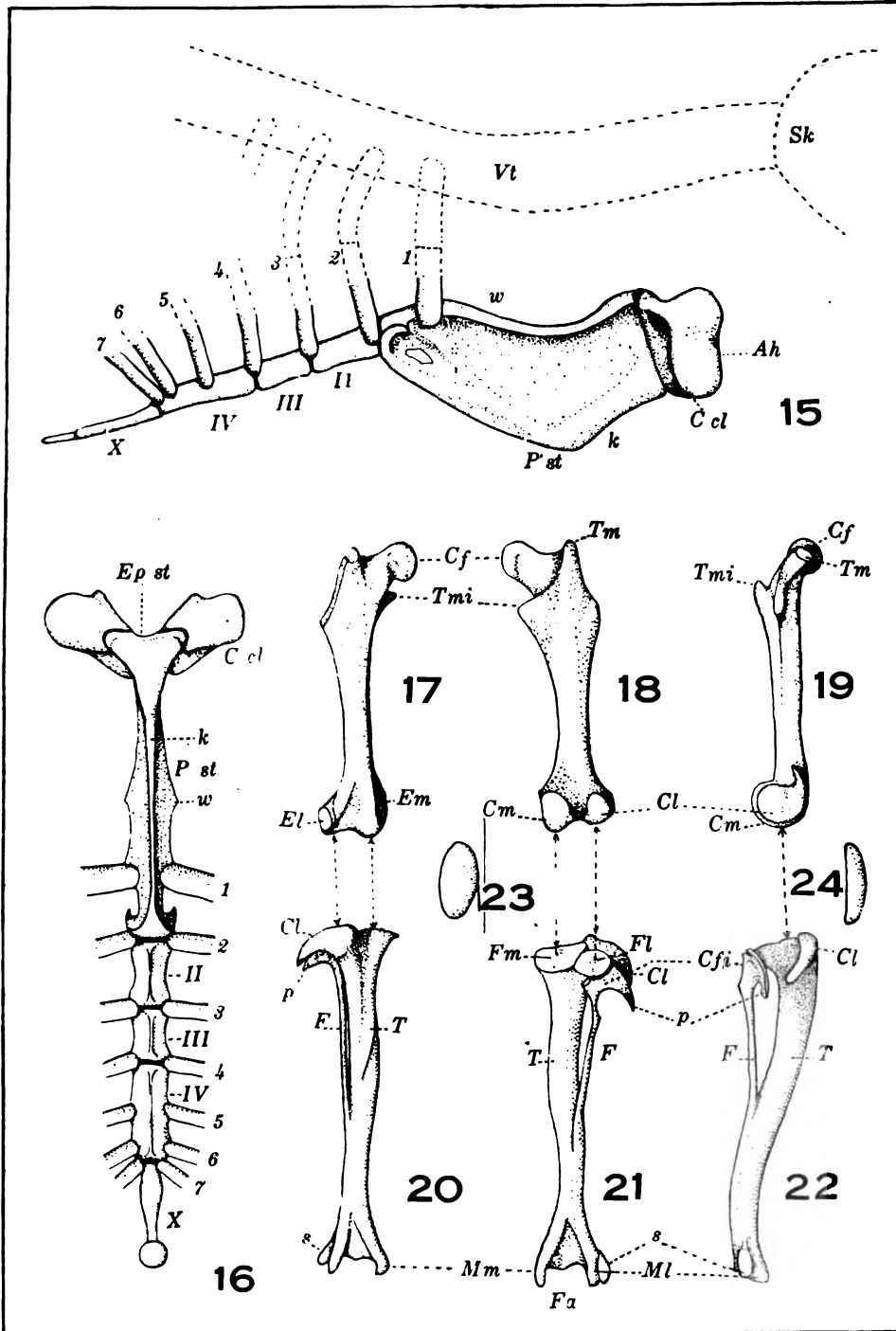
22 Enlarged lateral view of the tibia (*T*) and fibula (*F*), from the lateral aspect.

23 Enlarged front view of the patella.

24 Enlarged lateral view of the patella, lateral aspect.

ABBREVIATIONS

Ah, facies articularis humeri; *C cl*, coracoclavicle; *Cf*, caput femoris; *C fi*, capitulum fibulae; *Cl*, condylus lateralis; *Cm*, condylus medialis; *El*, epicondylus lateralis; *Em*, epicondylus medialis; *Ep st*, episternum; *F*, fibula; *Fa*, facies articularis inferior; *Fl*, facies articularis superolateralis; *Fm*, facies articularis superomedialis; *k*, keel of manubrium; *Ml*, malleolus lateralis; *Mm*, malleolus medialis; *p*, process from the head of the fibula; *P st*, manubrium (presternum); *s*, fibrilar sesamoid; *Sk*, skull; *T*, tibia, *Tm*, trochanter major; *Tmi*, trochanter minor; *Vt*, outline of vertebral column; *X*, processus siphoides; *1* to *7*, first to seventh ribs. The dotted lines connecting figures 17, 18, and 19 with figures 20, 21, and 22, respectively, indicate corresponding articular surfaces at the knee-joint. All figures are drawn with the same magnification.



Resumen por el autor, Mitchel Carroll.
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Una extra-diada y una extra-tetrada en la espermatogénesis de *Camnula pellucida* (Ortópteros); variaciones numéricas en el complejo cromosómico de un mismo individuo.

En cinco individuos, el número de cromosomas era inconstante dentro de la misma gonada, a causa de la presencia de un supernumerario de valencia variable. En un mismo individuo puede faltar el extra-cromosoma en algunos complejos, ser impar en algunos, par en otros y hasta aparecer triplicado. Si es impar, el cromosoma supernumerario pasa indiviso generalmente a una de las células hijas producidas durante la primera mitosis de maduración, segregándose libremente con relación al cromosoma accesorio y dividiéndose lo mismo que este durante la segunda mitosis de maduración. Por excepción el supernumerario impar se divide durante la primera mitosis; en este caso las extra-mónadas pasan indivisas a una u otra de las espermátidas durante la segunda mitosis. Si son pares los extra-homólogos se unen en la sinapsis, como cualquier par de eucromosomas, durante la primera generación de espermátocitos para formar una tetrada típica, que se comporta como las otras tetradas. Si es triple, dos de los extra-elementos se unen por sinapsis durante la primera mitosis, mientras que el tercero permanece libre y se segrega independientemente. La valencia variable del supernumerario es probablemente el resultado de la falta accidental de separación de sus elementos homólogos durante la mitosis. El autor ha encontrado varios casos de falta de separación. Los cinco individuos que poseen extra-cromosoma son de la misma localidad. Como dos de ellos fueron obtenidos en 1909 y tres en 1915 es posible que el supernumerario se haya transmitido durante varios años en esta raza. Dicho elemento podría tener indudablemente una influencia importante sobre las proporciones mendelianas y suministrar un mecanismo para la producción de mosaicos.

Translation by José F. Nonidez
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AN EXTRA DYAD AND AN EXTRA TETRAD IN THE SPERMATOGENESIS OF CAMNULA PELLUCIDA (ORTHOPTERA); NUMERICAL VARIATIONS IN THE CHROMOSOME COMPLEX WITHIN THE INDIVIDUAL

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FOURTEEN PLATES (ONE HUNDRED AND THIRTY-NINE FIGURES)

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INTRODUCTION

A. Scope of the paper

The investigation, of which this paper is a preliminary report, is one of a series based upon a large collection of orthopteran material brought together by Doctor McClung for the study of chromosome behavior. The present investigation deals with the chromosomes of the genus *Camnula*, and derives its chief value from its relation to the preceding studies of the series. It is not yet possible to report on the chromosomes of a representative series of the genus. But the work has disclosed two phenomena of special interest. These are: the conjugation during maturation of a homologous pair of extra chromosomes, or supernumeraries, and clear-cut, indisputable instances of definite numerical variations within the individual, in germinal chromosome complexes in non-pathological tissue. This paper is confined mainly to a consideration of these unusual conditions. Since, however, the camnulan chromatoid body differs somewhat in its behavior from similar structures hitherto reported, a short account is given of this element.

B. Material and acknowledgments

The genus *Camnula* is a member of the subfamily Oedipodinae of the orthopteran family Acrididae. But one species, *pellucida*, is recognized by taxonomists. This species inhabits the North American continent from the Atlantic to the Pacific. In the higher mountains it is found southward to Pennsylvania, elsewhere in the East it is confined to New England and Canada. In California it occurs in the San Joaquin-Sacramento Valley, and thence northward to British Columbia. In the Rocky Mountain region it penetrates southward to New Mexico and Arizona, and in the interior plains to Nebraska and Illinois. The northern limits of its range are not known.

The species, according to Mr. J. A. G. Rehn, of the Academy of Natural Sciences of Philadelphia, is very plastic and greatly influenced by its environment, in color, size, and less decidedly in certain details of its external anatomy.

The material on which this report is based consists of the testes of ten individuals from certain islands in Puget Sound. Individuals 950, 951, 954.1, and 980 were collected by Doctor McClung in 1909. The remainder, 2482, 2503, 2511, 2518, 2525, and 2526, were secured by Doctor Carothers during the summer of 1915.

In addition to these animals, there is a considerable amount of material in Doctor McClung's collection from the Puget Sound region and from various other sections of the United States and Canada. I have gone over much of this material, making random counts in the customary manner, without discovering anything unusual in chromosome number or behavior. But a detailed study was made of only the ten individuals mentioned above, and we are concerned here chiefly with the peculiar conditions, seemingly unique among cytological phenomena, found in five of these ten animals.¹

I am indebted to Dr. C. E. McClung and Dr. E. Eleanor Carothers, of the University of Pennsylvania, for the use of the material, for much valuable instruction in cytological methods, and for giving so generously of their time in checking up many of my observations. It is only fair to them to state, however, that the nature of the phenomena described and the amount of statistical matter presented in this paper are such that mistakes or imperfections in the work might easily escape detection by even such able cytologists.

Dr. P. P. Calvert, of the University of Pennsylvania, and Mr. J. A. G. Rehn, of the Academy of Natural Sciences of Philadelphia, have kindly furnished me with information concerning the taxonomy and distribution of *Camnula pellucida*. Mr. Rehn confirmed the identification of nine of the ten animals used in this investigation. (The tenth specimen, 950, was lost.)

Miss Ruth McClung was good enough to prepare for me some transverse sections of a camnulan testis with which to check my counts of the follicles.

¹ Some years ago Doctor McClung, in a preliminary survey of a number of species, noticed some peculiarity about the complexes of specimen 950; but when the present study was begun it was not suspected that variations in the chromosome number occurred within the individual.

C. Technique and nomenclature

Individuals 2482, 2503, 2511, 2518, 2525, and 2526 were killed with xylol. The killing agent for each animal collected in 1909 was not recorded. But as only xylol and cyanide were used, it is evident, from what has since been learned of the effect of different killing agents (McClung, '18), that 954.1 and 980 were killed with cyanide and 950 with xylol. In the former two the spindles are short, the chromosomes crowded and contracted; in the latter, the spindle is long, the chromosomes extended and widely spaced.

The fixative for all testes was strong Flemming solution. The sections on slides 2482 to 2526, inclusive, prepared by the writer, were cut either 8 or 10 μ in thickness. The slides of individuals 950, 951, 954.1, and 980 were prepared at the University of Kansas, and the sections are 7 μ in thickness. The thin sections require more time for study on account of the greater difficulty of matching, but are often helpful when examining particular chromosomes.

Flemming's tricolor and Heidenhain's iron-hematoxylin were used for staining.

All terms employed in this paper have long been in use by students of orthopteran spermatogenesis and are those, in the main, proposed by McClung in early papers of this series (McClung, '00, '05, '14).

THE TESTES

The acrididian testis has been described and figured by Sutton ('00, '02), Davis ('08), and Robertson ('16). In all essential features the testis of *Camnula* agrees with the descriptions and illustrations of this organ in other species of the family as given by the above authors. But there are certain mathematical details in the gross and histological structure of the camnulan testis which must be mentioned in order that the probable error in the chromosome statistics given beyond can be appreciated.

The testes are united into a single mass in the manner typical of the family. Each testis is composed of a number of cylindrical follicles. No mention is made in the literature of the number of

follicles in a testis. Counts made in eight individuals indicate that the number for each testis in *Camnula* is in the neighborhood of thirty or sixty for the whole organ.

Each follicle (Sutton, '00, fig. 1; Davis, '08, fig. A; Robertson, '16, plate III) is enclosed in a membrane of connective tissue and is connected at its cephalic or proximal end with the vas deferens. In section there can usually be seen at the distal end the apical cell surrounded by the primary spermatogonia. The secondary spermatogonia arise by mitotic division from the primary spermatogonia in the usual manner. The former can be distinguished from the latter by their more proximal position in the follicle and by the fact that they are arranged in groups or cysts, each cyst being surrounded by an investment of connective tissue.

All the secondary spermatogonia within a cyst are, of course, descendants of a single cell. Sutton is the only student of acrididian spermatogenesis who gives the definitive number of secondary spermatogonia for each cyst. He estimates that in *Brachystola magna* the number is 256 (Sutton, '00, '02). In *Camnula* there seems to be invariably 64. Since each of these becomes transformed into a first spermatocyte, the number of first spermatocytes within a cyst is also 64. Hence there are 128 second spermatocytes in each cyst and 256 spermatids.

THE CHROMATOID BODY

Chromatoid bodies, or similar granules, have been described for the rat by Lenhossek ('98), Duesberg ('08), Regaud ('10), and Allen ('18), and for the Crustacea by Fasten ('14, '18). Among insects such bodies have been described by Wilson for the Hemiptera ('13); by Lewis and Robertson ('16), Payne ('16), and Plough ('17) for the Orthoptera.

In the main the cytoplasmic body which is present in the germ cells of *Camnula* behaves like similar structures described by Wilson ('13) for *Pentatoma*, and Plough ('17) for *Rhomaleum*. Unlike the body in *Rhomaleum*, however (Wilson gives an account of its behavior in only a single individual of *Pentatoma*), its behavior is not constant for the species. In some individuals, as

950 and 2482, it seems to have the same history as in *Pentatoma* and *Rhomaleum*, appearing in the growth stages as a large body staining like chromatin and lying in the cytoplasm, though often pressed against the nuclear membrane. In individuals of this type it is usually a conspicuous body also at the first maturation division (*c* in figs. 46 and 48), but smaller similar staining granules may be present in the same cells (*c* in figs. 46 and 48). The latter I have called 'chromatoid granules.' One at least of these granules, which are sometimes seen in the cytoplasm, may be intranuclear in origin; for a small chromatic spheroid is often seen within the nucleus at diakinesis, while the nuclear membrane is still intact. A possible source of this spheroid may be seen at *c*, figure 47, plate 5, where a granule or 'chromomere vesicle' (Carothers, '17) is connected with a spermatogonial chromosome by a thin strand. The rest, judging from conditions observed in other individuals, may be homologues of the larger body. The large body has never been seen to divide (though I have observed it in a few instances apparently breaking up at the first spermatocyte kinesis). It usually remains passive in the cytoplasm, and consequently at the end of the first mitosis is found in but one-half of the second spermatocytes. It starts to disintegrate, usually in the second spermatocytes, and when seen during the second mitosis is much smaller than in the first. Occasionally, however, it may be as large even in the spermatids as in the first spermatocytes. The substance of the body seems to pass into the tail of the developing spermatozoon and to be extruded as described by Wilson and Plough.

Rarely in the above individuals two nearly equal chromatoid bodies can be seen in the same first spermatocyte.

In individual 954.1 a conspicuous chromatoid body is present in the growth stages, but almost invariably fragments before the first maturation division. In a few cells (fig. 15, *c*) a small chromatic body was observed in the metaphase, in addition to the usual chromosomes. This element lies in the spindle and appears more like a chromosome fragment than a chromatoid body. It is too small to be identified with the extra chromosome to be described presently.

In individual 2511 a moderate-sized chromatoid body can often be seen in the cytoplasm, at about the pachytene stage. Small bodies can sometimes be found, too, in the cytoplasm of germ cells of 2525 at this stage. In individuals of this type a single conspicuous body is never seen in the metaphase of the first mitosis, but chromatoid granules are often scattered about through the cytoplasm in both the first and second divisions (*c* in figs. 65, 68, 106, 108, 109, 114, 117).

Sometimes, as in individual 2526, there is never present a chromatoid body as such, but several chromatoid granules are usually observed in all cells after the zygotene stage (*c* in figs. 134, 137, 138, 139).

In individuals 2511, 2525, and 2526, since the chromatoid granules are scattered about at random through the cytoplasm at the first and second divisions, it is evidently impossible for these granules to be distributed to one-fourth of the spermatids in the exact manner that occurs in the case of the chromatoid bodies in *Pentatoma* (Wilson, '13) and *Rhomaleum* (Plough, '17).

One cannot be sure in individuals like 980, which exhibit a reversed staining reaction (McClung, '18), if chromatoid granules are present or absent. The cytoplasm is darkly stained and highly granular. There is certainly no single large chromatoid body present.

Granules staining like chromatin can sometimes be seen in the apical cell, primary spermatogonia, and early secondary spermatogonia (*c*, fig. 49). These are probably the same as the 'neutral red granules' of Lewis and Robertson ('16) and Plough ('17), which Plough identified with the chromatoid body.

The origin of the chromatoid body in *Camnula* I have not attempted to work out. This, if it appears worth while and feasible, can be better undertaken in connection with some breeding experiments which it is hoped to start in the future. Where there is such variation between individuals in regard to this structure, it would seem better to know the genetics of the case before undertaking an extended investigation.

THE NORMAL CHROMOSOME COMPLEX

Excluding the Tettigidae, which apparently do not belong with this group, the observations of over twenty different investigators on more than forty genera and sixty species of the Acrididae have shown that the diploid number of chromosomes in the males of this family is almost uniformly twenty-three and the haploid number twelve (McClung, '14, '17; Harvey, '16).

Apparent reductions in number in *Hesperotettix*, *Mermiria*, and *Chortophaga* have been demonstrated by McClung ('05, '14, '17) to be due to the formation of multiples. Robertson ('16) has shown that the same phenomenon is in all probability responsible for the apparent reduction in *Chorthippus* (*Stenobothrus*). Doctor Carothers has some unpublished data of a similar character for *Circotettix*. Careful study may bring the other two seemingly aberrant forms, *Chloealtis* (McClung, '14, '17) and *Pamphagus* (Granata, '10), into line with the numerical characteristics of the acrididae chromosome complex.

Some individuals of certain species, *Trimerotropis fallax* and *Circotettix lobatus* (Carothers, '17), *Trimerotropis suffusa* (*fallax*?) (Wenrich, '17), *Herperotettix viridis* (McClung, '17), have one or two elements in addition to the typical diploid and haploid number. These additional elements are the supernumeraries. They differ from the other chromosomes in that they are not essential constituents of the complex. The other elements, either singly or as parts of multiples, are always present in every germ cell up to and including the first spermatocytes of every individual of the species, genus, and family (with the doubtful exceptions noted above); the supernumeraries may or may not be present in the cells of different individuals of a species.

McClung ('14) reported the diploid number in the males of *Camnula pellucida* to be twenty-three, and the haploid number twelve. In view of the fact, as has just been pointed out, that these are the numbers which are found practically throughout the family and that counts by the writer in some thirty-four individuals from various sections of the United States and Canada indicate that in the spermatogonia twenty-three elements and

in the first spermatocytes twelve elements are always present, it seems justifiable to regard twenty-three and twelve as the fundamental numbers for the male germ cells of this genus.

Since, nevertheless, numerical variations in the organization of the chromosome complex occur within the individual in the species *pellucida*, it is impossible to state, without having made systematic counts in every cyst containing mitotic figures, what the conditions are in any given animal. All possible chromosome counts can be made, for instance, in the cells of nine consecutive first spermatocyte cysts of individual 2525 (table 3) before discovering any variation, and yet the number of elements in the cells of this generation varies from twelve to thirteen. Furthermore, the valence of the thirteenth element varies: in some cysts it is a dyad, in others a tetrad. Again, in individual 2526 counts were made in every cyst and follicle in which metaphase figures occurred without discovering any variation. But an examination of some first spermatocyte prophase showed the chromosome count to be inconstant.

The fundamental diploid or spermatogonial metaphase complex in *Camnula* consists, then, of twenty-three dyads; and the haploid or first spermatocyte complex of eleven tetrads and one dyad, the accessory. There are the usual two classes of second spermatocytes with respectively eleven and twelve dyads.

I have observed nothing unusual about these chromosomes. They have terminal fiber attachments and consequently are of the *Hippiscus* (McClung, '14) or telomitic (Carothers, '17) type. Their form and behavior have been fully discussed by McClung ('14), Wenrich ('16), Carothers ('17), and others.

Plate 2, illustrating the complexes of individual 954.1, and plate 5, illustrating those of no. 2482, will serve to represent the fundamental chromosomal organization for the various germ-cell generations in the *camnulan* testis.

Individual 954.1 was collected on Orcas Island, Puget Sound, Washington, in 1909. Nothing unusual was found in the germ cells of this animal except the element labeled *C* in figure 15 (pl. 2). This element has been identified tentatively as the chromatoid body. It was observed in only three or four meta-

phase cells and might possibly be a chromosome fragment. No other departures from the numerical organization characteristic of the acrididae complex were observed in over two hundred and sixty metaphases examined. These two hundred and sixty cells consisted of: two hundred first spermatocytes, distributed among sixteen cysts and thirteen follicles; fifty-nine second spermatocytes divided among four cysts and three follicles, and one spermatogonial metaphase (the only one in the testis suitable for a count).

An inspection of plate 5 will show that individual 2482 is also apparently perfectly regular with respect to its chromosome organization. This animal was collected in 1915 on San Juan Island, Puget Sound, Washington. From five to twenty (usually ten or twelve) counts were made in each of fifteen first spermatocyte cysts and from one to three counts in each of eight spermatogonial cysts. These twenty-three cysts were distributed among twenty-one follicles. There were no second spermatocytes containing mitotic figures in this testis.

Systematic counts in individuals 951 (collected on Orcas Island, Puget Sound, in 1909) 2503, and 2518 (both collected on San Juan Island, Puget Sound, in 1915) failed to reveal anything unusual or irregular in the numerical organization of the chromosome groups of these animals. No conditions were found differing in any respect from the complexes illustrated on plates 2 and 5. The figures on these plates represent, in fact, the typical conditions in the material I have examined thus far.

CHROMOSOME GROUPS OF ABERRANT INDIVIDUALS

A. The counts from atypical animals

Striking variations in the chromosome complex were first discovered in individual 950. This animal was collected on Orcas Island, Puget Sound, Washington, in 1909. Soon afterward the testis was sectioned and mounted, but not studied in detail until it was loaned to me a couple of years ago. For the reason that the slides are in poor condition, many sections of the testis being missing, an analysis of the chromosome counts is not possible.

Hence no attempt was made to get a complete series of counts. The results in fifteen cysts are as follows:

First spermatocytes

Follicle A } 18 metaphases: 11 tetrads + 1 accessory + 1 dyad (figs. 7 and 8).
Cyst 1 } 1 metaphase: 10 tetrads + 1 accessory + 3 dyads (fig. 10).²

Follicle B } 9 metaphases: 12 tetrads + 1 accessory (figs. 1, 2, 3).
Cyst 3 } 1 anaphase: 12 dyads + 13 dyads.

Follicle C } 6 metaphases: 11 tetrads + 1 accessory + 1 dyad.
Cyst 4 }

Follicle D } 7 metaphases: 12 tetrads + 1 accessory.
Cyst 10 }

Follicle E } 5 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 9).
Cyst 5 }

Follicle F } 2 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 6).
Cyst 6 }

Follicle G } 6 metaphases: 12 tetrads + 1 accessory (fig. 4).
Cyst 7 }

Follicle H } 5 metaphases: 11 tetrads + 1 accessory + 1 dyad.
Cyst 8 }

Follicle I } 3 metaphases: 12 tetrads + 1 accessory (fig. 5).
Cyst 9 }

(In the above complexes the accessory or sex chromosome is, of course, a dyad; it is listed separately from other elements of like valence throughout the first spermatocyte statistics given in this paper because of its differential character, it being always easily recognizable.)

² In this cell the two dyads of tetrad number 10 either failed to synapse or divided prematurely. One of these dyads has gone to one pole, while the other is still in the equatorial plate. If both had gone to the same pole as the small dyad (s), a second spermatocyte cell containing two extra dyads would have resulted. If both had gone to the opposite pole, a second spermatocyte cell containing a large extra dyad would have been the result. In either case we would have had also a second spermatocyte cell lacking dyad number 10.

Second spermatocytes

Follicle K } 4 metaphases: 12 dyads.
 Cyst 12 } 3 metaphases: 13 dyads.

Follicle L } 4 metaphases: 11 dyads.
 Cyst 13 } 2 metaphases: 12 dyads:.

Follicle M } 3 metaphases: 11 dyads.
 Cyst 14 } 3 metaphases: 12 dyads.
 } 3 metaphases: 13 dyads.

Spermatogonia

Follicle N } 2 metaphases: 25 dyads.
 Cyst 15 }

Follicle O } 1 metaphase: 23 dyads.
 Cyst 16 }

Follicle P } 1 metaphase: 23 dyads.
 Cyst 17 }

(These were the only spermatogonial mitoses which gave clear counts.)

Thus, in this individual, there is a difference in the chromosome counts of the first spermatocyte metaphase of one dyad. In twenty-six cells contained in four different cysts (nos. 3, 7, 9, and 10) and four follicles (B, G, I, and D) there are present twelve tetrads and one dyad, the accessory (figs. 1, 2, 3, 4, and 5 of pl. 1). In each of thirty-seven cells, divided among five cysts (nos. 1, 4, 5, 6, and 8) and five follicles (A, C, E, F, and H), the complex consists of eleven tetrads and two dyads, one of which is the accessory (figs. 6, 7, 8, 9, and 10 of pl. 1).

In addition to the two classes of second spermatocytes, containing, respectively, eleven and twelve dyads, typical of the Acrididae, there is present a thirteen dyad class (follicles K and M).

There are at least two classes of spermatogonia, those containing the normal number of chromosomes (cysts 16 and 17) and those containing twenty-five dyads (cyst 15).

Chromosome counts were made in every cyst containing cells (of whatever generation) with mitotic figures in each of the four

individuals still to be considered. The results of these counts in nos. 980, 2511, and 2525 are given in the form of tables. It is to be understood that, though the valence of the accessory is not given in the case of the first spermatocytes, it is always a dyad in this cell generation and passes to one of the poles undivided, as a V, during the anaphase. Where only two or three counts are given for a particular cyst, as is usually the case for the spermatogonia, it is to be understood, also, that it was not possible to secure more of a trustworthy character. This was sometimes the case (as in the spermatogonia), because the cyst contained but a few cells. First and second spermatocyte cysts often contain cells in different phases; hence, though there are sixty-four cells in the former and one hundred and twenty-eight in the latter, it is sometimes possible to make but two or three counts.

In the tables the counts have been arranged like the counts just given for 950, by cyst and follicle. The cysts are numbered consecutively in the order in which they were examined. The follicles are denoted by letters and are represented by the horizontal columns. The vertical columns represent the three classes of germ cells: spermatogonia, first spermatocytes, and second spermatocytes. Since the results of the counts in each cyst are placed in the proper columns horizontal and vertical, all the counts of a given cell generation or of a particular follicle may readily be compared. (Table 1, p. 389.)

In individual 2526 (from San Juan Island, 1915) counts were obtained in eight spermatogonial cells from five cysts, one hundred and seventy-one first spermatocyte cells distributed among ten cysts, and forty-four second spermatocytes from eight cysts. These twenty-three cysts (all in which mitotic figures occur) are contained in twenty different follicles. The chromosome count is constant for these twenty follicles. The spermatogonial complexes consist of twenty-five dyads each, the first spermatocytes of twelve tetrads and one dyad (the accessory). Two classes of second spermatocytes were found, containing respectively twelve and thirteen dyads. Representative complexes of all three cell generations are illustrated on plates 13 and 14.

I at first thought that the count was probably constant for this animal, but on examining some growth stages and first spermatocyte prophase, a precocious dyad (in addition to the accessory) was discovered in two cysts. Although it was difficult to secure accurate counts in these cysts, I think each complex consisted of eleven tetrads plus the accessory, plus one dyad.

B. Analysis of the counts

In the germinal complexes of each of five (nos. 950, 980, 2511, 2525 and 2526) atypical individuals, there is constancy neither in the number of chromosomes nor in the amount of chromatin.

It is evident (counts given for no. 950, tables 1, 2, 3, and pls. 1, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, and 14) that the variations in the composition of the chromosome group are due to the presence of extra or supernumerary elements. No complex was found, which has less than the normal number of chromosomes for its cell generation.

In all spermatogonial metaphases there are at least twenty-three dyads constantly present; in all first spermatocyte metaphases there are (with one apparent exception, illustrated in fig. 10, due to a premature division or failure of pair no. 10 to synapse) at least eleven tetrads and one accessory (a dyad); there are never less than eleven dyads in a second spermatocyte metaphase. These are the numbers characteristic of the several germ-cell generations of the animals which manifest no departure from the typical or normal chromosomal organization (pls. 2 and 5).

The numerical variations in the spermatogonia are due to the presence of from one to three dyads in excess of the typical number. Variations in the first spermatocyte counts are also clearly due to extra elements which may be for any given complex, a dyad, a tetrad, or a dyad and a tetrad. (In three cells there are two extra dyads present.)

In the second spermatocyte metaphases the departures from the normal numerical organization are the result of the presence of from one to possibly three extra dyads. Although in each of two cells (figs. 114 and 117), to be discussed later, the aberrant count is due to the presence of a monad.

TABLE 1

Chromosome counts in individual 980

Collected in 1909. Locality: San Juan Island, Puget Sound

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
A		<i>Cyst 1</i> 17 metaphases: 12 tetrads + 1 accessory (figs. 27, 28)	
B		<i>Cyst 2</i> 6 prophases: 12 tetrads + 1 accessory (fig. 29)	
C		<i>Cyst 3</i> 17 metaphases: 11 tetrads + 1 accessory + 1 dyad (figs. 24, 32)	
		<i>Cyst 4</i> 15 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 26)	
D		<i>Cyst 5</i> 19 metaphases: 12 tetrads + 1 accessory	
		<i>Cyst 6</i> 13 metaphases: 12 tetrads + 1 accessory (fig. 30)	
E	<i>Cyst 18</i> 3 metaphases: 26 dyads (fig. 41)		<i>Cyst 7</i> 6 metaphases: 13 dyads (fig. 38) 2 metaphases: 14 dyads (fig. 35) 2 metaphases: 12 dyads 1 anaphase: 13 monads + 13 monads <i>Cyst 8</i> 16 metaphases: 13 dyads 7 metaphases: 12 dyads (fig. 37) 5 metaphases: 14 dyads 2 metaphases: 11 dyads

TABLE 1—Continued

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
F		<i>Cyst 9</i> 10 prophases: 12 tetrads + 1 accessory 2 metaphases: 12 tetrads + 1 accessory	
G		<i>Cyst 10</i> 21 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 25)	
H	<i>Cyst 20</i> 2 metaphases: 25 dyads (fig. 40)	<i>Cyst 11</i> 12 metaphases: 12 tetrads + 1 accessory	
		<i>Cyst 16</i> 6 metaphases: 12 tetrads + 1 accessory	
I		<i>Cyst 12</i> 20 metaphases: 11 tetrads + 1 accessory (figs. 21, 22, 31)	
J	<i>Cyst 19</i> 1 metaphase: 25 dyads (fig. 42)		<i>Cyst 13</i> 3 metaphases: 11 dyads 15 metaphases: 12 dyads (fig. 39) 5 metaphases: 13 dyads (figs. 34, 36)
K		<i>Cyst 14</i> 10 prophases: 11 tetrads + 1 accessory (fig. 23)	
L		<i>Cyst 15</i> 11 metaphases: 12 tetrads + 1 accessory	
M	<i>Cyst 21</i> 2 metaphases: 25 dyads	<i>Cyst 17</i> 11 metaphases: 12 tetrads + 1 accessory (fig. 33)	

TABLE 1—*Continued.**Numerical classes of metaphase complexes found in 980*

2 classes of spermatogonia.....	$\left\{ \begin{array}{l} 25 \text{ dyads} \\ 26 \text{ dyads} \end{array} \right.$
3 classes of first spermatocytes....	$\left\{ \begin{array}{l} 11 \text{ tetrads} + 1 \text{ accessory} \\ 11 \text{ tetrads} + 1 \text{ accessory} + 1 \text{ dyad} \\ 12 \text{ tetrads} + 1 \text{ accessory} \end{array} \right.$
4 classes of second spermatocytes..	$\left\{ \begin{array}{l} 11 \text{ dyads} \\ 12 \text{ dyads} \\ 13 \text{ dyads} \\ 14 \text{ dyads} \end{array} \right.$

While the chromosome count in the atypical individuals is not constant for the gonad, within the subdivisions of this organ the organization of the complex does not seem to vary.

No numerical variations in the metaphase complexes have appeared anywhere in these counts within a first spermatocyte cyst.³ With one possible exception in individual 2511, the same is true for the spermatogonial complexes. The exception occurs in cyst 13, follicle K, of 2511 (table 2). In this cyst it was only possible to make two counts. One (pl. 7, fig. 63) clearly gave twenty-five dyads, the other complex was interpreted as probably consisting of twenty-six dyads; I am not sure enough of the interpretation, however, to consider this cell an exception to what appears to be a rule. In the case of the spermatogonia and the first spermatocytes, then, no diversities in the chromosomal organization have been found within the cyst in the animals so far examined. The counts are always the same for all the cells of a cyst of either of these generations.

Similarly, as an examination of the data given above for the aberrant animals will show, the organization of the second spermatocyte chromosome groups is typically consistent within the cyst. Since the accessory passes undivided to one pole at the first

³ The telophase cell illustrated in figure 108, which has one less dyad than the other cells in the cyst, is not necessarily an exception to the rule of constancy in number for the cyst, as the missing element might have been pulled out of place by the microtome knife.

TABLE 2

Chromosome counts in individual 2511

Collected in 1915. Locality: San Juan Island, Puget Sound

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
A	<i>Cyst 2</i> 3 metaphases: 23 dyads		<i>Cyst 1</i> (counts uncertain) 5 prophases: 11 dyads 11 prophases: 12 dyads 3 prophases: 13 dyads
B	<i>Cyst 3</i> 6 metaphases: 23 dyads		
	<i>Cyst 4</i> 6 metaphases: 23 dyads		
C			<i>Cyst 5</i> 2 metaphases: 11 dyads (figs. 65) 16 metaphases: 12 dyads (fig. 66) 16 metaphases: 13 dyads (figs. 68, 70, 71) 1 metaphase: 14 dyads (fig. 67)
D	<i>Cyst 6</i> 3 metaphases: 26 dyads (fig. 64)		
E		<i>Cyst 7</i> 17 metaphases: 12 tetrads + 1 accessory (figs. 57, 58, 59, 60, 61)	
F	<i>Cyst 8</i> 1 metaphase: 23 dyads		
G			<i>Cyst 9</i> 3 metaphases: 11 dyads 4 metaphases: 12 dyads

TABLE 2—Continued

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
H			<i>Cyst 10</i> 7 metaphases: 12 dyads 4 metaphases: 13 dyads (fig. 69) 2 anaphases: 12 monads + 12 monads 1 anaphase: 13 monads + 13 monads
I		<i>Cyst 11</i> 8 metaphases: 12 tetrads + 1 accessory + 1 dyad (figs. 52, 53, 54, 55 56)	
K	<i>Cyst 13</i> 1 metaphase: 26 dyads 1 metaphase: 25 dyads (fig. 63)		
L	<i>Cyst 14</i> 2 metaphases: 23 dyads (fig. 62)		
M		<i>Cyst 15</i> 2 early prophase: 11 tetrads + 1 accessory	

Numerical classes of metaphase complexes found in 2511

3 classes of spermatogonia.....	{ 23 dyads 25 dyads 26 dyads
3 classes of first spermatocytes....	{ 11 tetrads + 1 accessory 12 tetrads + 1 accessory 12 tetrads + 1 accessory + 1 dyad
4 classes of second spermatocytes..	{ 11 dyads 12 dyads 13 dyads 14 dyads

TABLE 3

Chromosome counts in individual 3525

Collected in 1915. Locality: San Juan Island, Puget Sound

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
A	<i>Cyst 2</i> 1 metaphase: 25 dyads (fig. 97)	<i>Cyst 1</i> 1 metaphase: 12 tetrads + 1 accessory (fig. 72) 2 anaphases: 12 dyads + 13 dyads (figs. 112, 73) 1 anaphase: 12 dyads + (11 dyads + 2 monads) (fig. 108) 1 anaphase: (13 dyads + 1 monad) + (11 dyads + 1 monad) (fig. 109)	
B		<i>Cyst 3</i> 15 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 74) <i>Cyst 22</i> 6 prophases: 11 tetrads + 1 accessory + 1 dyad (fig. 119)	
C		<i>Cyst 4</i> 25 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 75)	
D		<i>Cyst 5</i> 21 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 76)	
E			<i>Cyst 6</i> 9 metaphases: 12 dyads (fig. 100) 8 metaphases: 13 dyads (fig. 103) 1 metaphase: 11 dyads 8 anaphases: 12 monads + 12 monads (figs. 113, 102) 4 anaphases: 13 monads + 13 monads (fig. 116, 104) 1 anaphase: 13 monads + 14 monads (fig. 115)

TABLE 3—Continued

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
F		<i>Cyst 7</i> 2 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 93)	
G		<i>Cyst 8</i> 20 metaphases: 11 tet- rads + 1 accessory + 1 dyad (fig. 78)	
H	<i>Cyst 10</i> 3 metaphases: 24 dyads (figs. 99, 101)	<i>Cyst 9</i> 20 metaphases: 11 tet- rads + 1 accessory + 1 dyad (figs. 77, 106) 1 anaphase: 12 dyads + 13 dyads	<i>Cyst 11</i> 2 metaphases: 12 dyads 2 metaphases: 13 dyads 1 anaphase: 13 monads + 13 monads 1 anaphase: 12 monads + 13 monads
I		<i>Cyst 12</i> 25 metaphases: 11 tet- rads + 1 accessory + 1 dyad (figs. 80, 81, 105)	
J		<i>Cyst 13</i> 20 metaphases: 11 tet- rads + 1 accessory + 1 dyad (fig. 79) 1 anaphase: 11 dyads + 13 dyads 1 anaphase (11 dyads + 1 monad) + (12 dyads + 1 monad) (fig. 111)	
K		<i>Cyst 14</i> 32 metaphases: 12 tet- rads + 1 accessory (figs. 94, 82, 83, 84, 86) 2 metaphases: 11 tetrads + 1 accessory + 2 dy- ads (fig. 85) <i>Cyst 15</i> 2 prophase: 12 tetrads + 1 accessory	

TABLE 3—Continued

FOLLICLE	SPERMATOGONIA	FIRST SPERMATOCYTES	SECOND SPERMATOCYTES
L		<i>Cyst 16</i> 28 metaphases: 11 tetrads + 1 accessory (figs. 95, 87, 88, 89, 90)	
M		<i>Cyst 17</i> 12 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 91)	
		<i>Cyst 25</i> 2 prophase: 11 tetrads + 1 accessory + 1 dyad (fig. 118)	
N		<i>Cyst 18</i> 2 metaphases: 11 tetrads + 1 accessory + 1 dyad (fig. 92) 2 anaphases: 12 dyads + 12 dyads (fig. 107) 1 anaphase: (12 dyads + 1 monad) + (11 dyads + 1 monad) (fig. 110)	
O			<i>Cyst 19</i> 2 metaphases: 11 dyads + 1 monad (figs. 114, 117)
Q	<i>Cyst 21</i> 2 metaphases: 24 dyads (figs. 96, 98)		

Numerical classes of metaphase complexes found in 2525

2 classes of spermatogonia.....	{ 24 dyads 25 dyads
4 classes of first spermatocytes...	{ 11 tetrads + 1 accessory 11 tetrads + 1 accessory + 1 dyad 12 tetrads + 1 accessory 11 tetrads + 1 accessory + 2 dyads ⁴
4 classes of second spermatocytes	{ 11 dyads 11 dyads + 1 monad 12 dyads 13 dyads

⁴ This class is essentially the same as the preceding one; two of the dyads apparently failed to synapse.

maturation division, at least two classes of cells are found in every second spermatocyte cyst. Where an extra dyad is present in some first spermatocyte cysts of a gonad, second spermatocyte cysts containing three numerical classes of complexes are to be expected. For a supernumerary dyad in *Camnula*, as is the case for all such elements, so far reported in the *Acrididae*, usually passes undivided into one of the daughter cells at the first maturation division, and whether it accompanies the accessory or not is a matter of chance.

It is usually not possible to secure counts in more than one cyst and one cell generation within a particular follicle. But tables 1, 2, and 3 show where such counts have been obtained, they are constant for the follicle prior to the second spermatocyte generation.

In individual 980 (table 1, pls. 3 and 4) counts have been obtained in several cysts within each of the following follicles: C, D, E, H, J, and M. In each of the follicles E, H, J, and M counts are given for two different cell generations. It will be noted that the first spermatocyte counts (follicles C, D, H) are constant for the follicle. In follicle H the metaphases of spermatogonial cyst 20 each contains twenty-five dyads (pl. 4, fig. 40). If the numerical organization is consistent for the follicle, and if the twenty-five dyads consist of twelve pairs of chromosomes plus the accessory, we should find in the first spermatocyte complexes twelve tetrads plus the accessory (a dyad); and this is exactly what is found in cysts 11 and 16 of this follicle. In follicle M the spermatogonial metaphases of cyst 21 consist of twenty-five dyads each and the expected first spermatocyte complexes are found in cyst 17 (plate 4, fig. 33). Spermatogonial complexes consisting of twelve pairs of dyads plus the unpaired accessory should give two classes of second spermatocyte metaphases, containing, respectively, twelve and thirteen dyads. This is what is found in follicle J. But there is recorded in addition three exceptional second spermatocyte complexes which are interpreted as containing eleven dyads each. All spermatogonial complexes consisting of twenty-six dyads so far found in *Camnula* seem to be made up of twelve pairs of chromosomes plus an un-

paired accessory plus an unpaired supernumerary. Numerically, in a follicle, as in E, giving a spermatogonial count of twenty-six, there should be three classes of second spermatocytes with, respectively, twelve, thirteen, and fourteen dyads. Furthermore, if, as is typical for supernumerary dyads in the Orthoptera (Carothers, '17; Robertson, '17), it is a matter of chance whether the undivided odd dyad of the first spermatocyte division accompanies the accessory into a daughter cell or not, the number of cells of the thirteen class should equal the sum of the cells of the other two classes. These conditions are fulfilled by cysts 7 and 8 of follicle E, except that, as in cyst 13, of follicle J, there are two counts of eleven recorded.

In 2511 (table 2, pls. 6 and 7), with the exception of follicles A and B, it was not possible to secure counts in more than one cyst of a follicle. The six counts obtained in spermatogonial cyst 3 of follicle B are identical with the six counts in cyst 4 of the same follicle. It was difficult to count the chromosomes in the prophases of second spermatocyte cyst 1, follicle A. Among the twenty counts recorded for this cyst, three, each containing thirteen dyads, are not in agreement with the twenty-three chromosome complexes of spermatogonial cyst 2 of this follicle. But it should be remembered that these are doubtful counts.

Counts are given for several cysts within each of the follicles A, B, H, K, and M of individual 2525 (table 3, pls. 8, 9, 10, 11, and 12). The metaphase complex and two of the four anaphase counts (pl. 8, figs. 72 and 73) in first spermatocyte cyst 1 of follicle A are in agreement with the spermatogonial count (pl. 10, fig. 97) in cyst 2 of the same follicle. The peculiar distribution of elements in the other two anaphases (figs. 108 and 109) will be discussed beyond (see 'non-disjunction of the supernumeraries'). The first spermatocyte metaphase complexes in cysts 3 and 22 of follicle B (figs. 74 and 119), in cysts 14 and 15 of follicle K (figs. 82, 83, 84, 86, and 94) and in cysts 17 and 18 of follicle M (figs. 91 and 118) are clearly constant in each case for the follicle. (As will be shown beyond, the two exceptions in cyst 14 are apparent and not real.) In Follicle H, I was fortunate in obtaining counts

in a spermatogonial, a first spermatocyte, and second spermatocyte cyst. The spermatogonial complexes (cyst 10; figs. 99 and 101) of twenty-four dyads are constituted as follows: eleven pairs of chromosomes plus the accessory plus a supernumerary dyad; this organization in the first spermatocyte metaphases (cyst 9, figs. 77 and 106) takes the form of eleven tetrads plus one accessory plus one extra dyad. The three expected classes of second spermatocytes for this follicle contain, respectively, eleven, twelve, and thirteen dyads. Since only six counts were secured in cyst 11, it is not surprising that the eleven-dyad class was not found. The anaphase with twelve monads going to one pole and thirteen to the other results from the fact that occasionally the odd dyad divides in the first, instead of the second mitosis.

The data so far presented, then, indicate: In five animals from islands in Puget Sound there is variation in the fundamental numerical organization of the germinal chromosome group within the individual. But with one possible exception in cyst 13 of individual 2511, the organization of the metaphase complex is constant for the cyst, save for the classes of second spermatocytes resulting from the customary segregation of elements in the first maturation division. The chromosome number is constant also for the follicle until after the first spermatocyte mitosis. The variations in the chromosome groups within the atypical individuals are due to the presence of an unpaired extra dyad, to a pair of extra dyads, or to both, in certain follicles of the gonads of these animals in addition to the elements of the normal complex.

C. The extra or supernumerary elements

In first spermatocytes there is usually no difficulty in distinguishing the supernumerary elements. A comparison of the complexes of normal individuals (pl. 2, figs. 11 to 14; pl. 5, figs. 43 to 45) and the normal chromosome groups (figs. 21, 22, 23, 31, 87, 88, 89, 90, and 95, pls. 3, 4, 9, and 10) in aberrant individuals, with the atypical complexes (figs. 1 to 10, 24 to 30, 32, 33, 52 to 61, 72 to 86, 91, 92, 120 to 130, pls. 1, 3, 4, 6, 8, 9, and

13) will show that the extra elements are those which have been placed in the column marked 'S.' The extra dyads (*S* in figs. 6 to 10, 24 to 26, 32, 74 to 81, 85, 91 to 93, 105, 106, 136) are always easily identified by their size, form, and position on the spindle. The supernumerary tetrad (*S* in figs. 1 to 5, 27 to 30, 57 to 61, 72, 73, 82 to 86, 120 to 130) is readily distinguished by its size and form from all other chromosomes except no. 3, and I believe it can ordinarily be distinguished from the latter.

In the spermatogonia, and usually in second spermatocytes, we have only the relative size of the extra elements as a criterion. Hence identification of the supernumeraries in the complexes of these two cell generations is often not absolute.

The extra dyads in the column labeled 'S' in plates 1 (figs. 6 to 10), 3 (figs. 24 to 26), 6 (figs. 52 to 55), 8 (figs. 74 to 81), 9 (figs. 85, 91, 92), 11 (figs. 105, 106), and 14 (fig. 136) are clearly homologues in size and form. The supernumerary tetrad (*S* in figs. 1 to 5, 27 to 30, 52 to 61, 82 to 86, 120 to 128) is, too, evidently composed of two of these homologous dyads.

It is true that slight differences in the apparent size of the silhouettes of both the extra dyads and extra tetrads in different complexes are discernible. But these variations are not constant even for the cyst and are no greater than those seen in other chromosomes, as, for instance, no. 3 or no. 4 (the accessory). Apparent differences in the size of homologous elements may be due to foreshortening or varying relations of the chromatids within the chromosomes (McClung, '14; Wenrich, '16, '17). Real differences in the size of genetically related elements can and do exist as in the tetrads with unequal homologues reported by Carothers ('13, '17), Voinov ('14), Wenrich ('16), and Robertson ('15). But such slight differences as may actually exist between these homologous supernumeraries in *Camnula* are not due to the loss of a specific part of the chromosome as in the above unequal pairs. They seem to be the result, rather, of varying degrees of condensation of the chromatin. For instance, the extra dyad in figure 79, plate 8, appears somewhat larger than the other supernumeraries of like valence for the reason that it has a constricted 'neck' and a knob on one end. This same

condition, to a less degree, can be seen in the supernumerary tetrad in figure 3, plate 1; figure 72, plate 8, and figure 135, plate 14. Euchromosome no. 3 exhibits similar but greater variations, as the plates reveal. Some of these variations in size are undoubtedly due to the technique employed.

All the supernumerary elements, then, in this material, whether paired or unpaired, when judged by the usual criteria of size and form, are evidently genetically related.

The extra dyad. Spermatogonial complexes containing unpaired supernumeraries are illustrated on plate 10 (figs. 96, 98, 99, 101). So far as I have been able to observe, such unpaired elements divide in the spermatogonial mitoses like any other chromosomes, as is presumably the case for all supernumeraries hitherto reported. For, since they are found in all the first spermatocyte cells of individuals in which they occur, all the extra chromosomes so far reported (Wilson, '05, '07 a, '07 b, '09 a, '09 b, '10; Stevens, '08, '12 a, '12 b; Carothers, '17; Wenrich, '17; McClung, '17; Robertson, '17) must divide in every spermatogonial mitoses. But when dealing with such variable material as *Camnula*, one is scarcely justified in making such a positive assertion without more extended observations than I have yet made.

In the postspireme stage of the first spermatocyte the unpaired element is somewhat precocious. It behaves in this respect like the accessory, condensing and contracting ahead of the euchromosomes (pl. 12, figs. 118 and 119). But it exhibits no tendency to synapse with the accessory.

In the first spermatocyte metaphase the unpaired extra dyad is a short rod and lies horizontally in the equatorial plate with one of its two chromatids directly above the other (pl. 3, fig. 26; pl. 8, figs. 79 and 80; pl. 9, fig. 92; pl. 10, fig. 93) as in a spermatogonial metaphase. A spindle fiber is typically attached at one angle (pl. 1, figs. 7 and 10; pl. 6, fig. 54), at doubtless the point of attachment for one of the fibers in spermatogonial mitoses. Usually the dyad passes undivided to one pole preceding the division of the euchromosomes (pl. 11, figs. 105, s, and 106, s). Rarely (pl. 8, fig. 79) spindle fibers are attached at both

the upper and lower angles of the proximal or synaptic end. It is when this occurs, no doubt, that the element divides, as it does occasionally, in the first maturation mitosis (pl. 11, fig. 110 s; pl. 12, fig. 111, s). Thus, for this element, the first maturation division is usually reductional, but may be equational. In this behavior it differs from the unpaired supernumeraries described by Carothers ('17) in *Trimerotropis* and *Circottetix*, Wilson ('07, '10) in *Metapodius* and *Banasa*, and Robertson ('17) in *Tettigidea*. In *Trimerotropis*, *Circottetix*, *Tettigidea*, and *Banasa* the supernumeraries are reported to pass always to one pole undivided in the first division, but to divide in the second. In *Metapodius* the unpaired elements invariably divide in the first and not in the second. Hence the camnulan extra dyad resembles more in its behavior at this stage the supernumeraries in *Ceuthophilus* and *Diabrotica*, which, according to Stevens ('08, '12 a, '12 b), divide indifferently in the first or second. The unpaired extra element when it fails to divide in the first mitosis segregates quite independently of the accessory. In figures 105 and 106 it is passing to the same pole as the accessory, in figure 107 it has gone to the opposite pole. It is a matter of chance whether it goes to one pole or the other. In this respect it is in agreement with most of the unpaired elements mentioned in the literature.

Every dyad present in a second spermatocyte cell divides at mitosis. Hence, although it is not possible to state positively that a particular element in a given second spermatocyte cell was an unpaired dyad which passed into one daughter cell undivided at the first spermatocyte mitosis, it is certain that all such dyads do divide at the second mitosis. Supernumerary dyads in second spermatocyte metaphase and anaphase are indicated by the letter *s* in figures 37, 38, 134, and 139.

When an extra dyad divides in the first mitosis it reappears in the succeeding metaphase of each daughter cell as a monad (pl. 12, figs. 114 and 117, s). In the anaphase of the second mitosis such a monad passes undivided into one of the daughter spermatids.

The supernumerary tetrad. A pair of dyads, the homologues of the unpaired element just described, is present in the spermatogonial complexes of a number of follicles in the atypical individuals (pl. 4, fig. 40, 42; pl. 7, fig. 63; pl. 10, fig. 97; pl. 14, figs. 131, 132, 133). These dyads behave in this generation and in the two succeeding ones like any typical euchromosome pair. In the late prophases of the first spermatocytes, unlike their unpaired homologue, they do not seem to be precocious when compared with the euchromosomes. They synapse like any other chromosome pair to form a typical tetrad which is not distinguished by any pronounced differential behavior. In the first spermatocyte spindle this tetrad appears usually as a rod, extended parallel to the axis of the spindle, but it may take the form of a V (figs. 52, S, 54, S) or a cross (figs. 2, 84, 127, S). In this mitosis, there is a slight apparent shift in the points of fiber attachment in the two dyads composing the tetrad when compared with their unpaired homologue. The fibers seem to be attached at the middle points of the polar ends of the two halves of the tetrad, instead of at one angle of the end as in the unpaired elements. The tetrad divides in metaphase (figs. 27, 72, 73, 112, 120, 121, 124, 125) in the typical fashion, the two halves passing to opposite poles as V's in anaphase. These halves divide like the rest of the dyads in the second spermatocyte mitosis (figs. 69, 70, 71, 104, 116, 138, 139).

A comparison of the extra tetrad and euchromosome no. 3 on plates 1, 2, 3, 6, 8, 9, 13, and figure 135 on plate 14 shows what a close resemblance there is between these two in size and form. In figures 3, 72, 120, and 135 there are even indications of the knobs, so often seen on the ends of no. 3, in the supernumerary tetrad. No. 3 in figures 11, 12, 13, and 22 could not be distinguished from the extra tetrad, were the latter present in these complexes. In figures 123, 124, and 125 I am not sure that the two elements have been properly identified. Yet, in spite of this close resemblance, it is possible, I think, in the majority of cells, to distinguish between these two chromosomes. All the chromosomes in 954.1 are more condensed, probably on account of the killing agent employed, than in the other animals. This fact

may account for the appearance of no. 3 in figures 11, 12, and 13, which is typical for the extra tetrad rather than for the former. The dumb-bell shape without knobbed ends (figs. 1, 4, 5, 28, 29, 30, 33, 53, 57, 82, 83, 121, 122, 123, and 128) or the form seen in figures 58, 59, 60, 61, 86, and 127, together with, it seems to me, a trifle smaller size than no. 3, can usually be taken as sufficiently diagnostic. Nor can I recall ever having certainly observed the extra element in the form of a ring as sometimes happens with no. 3 (figs. 129, 130).

I have not attempted to test no. 3 and the supernumerary tetrad metrically, because the difference between these two is small enough to fall within the limits of error in the drawings. Then, too, it should be possible to demonstrate more conclusively by a study of the early growth stages and by breeding the animals, if these two elements are genetically related. It is obvious that if the extra tetrad is a duplicate of a member of the normal chromosome group, that member is no. 3.

The triploid extra homologues. In a few follicles, 980 E and 2511 D and I, there are three extra dyads present. Spermatogonial metaphases of complexes containing these three are shown in figure 41, plate 4, and figure 64, plate 7. In the first spermatocyte metaphases (pl. 6, figs. 52, 53, 54, and 55) two of these dyads synapse to form a typical tetrad, while the third remains free. The latter passes undivided to one or the other pole. In the drawings, if it is going toward the same pole as the accessory, it is placed above the extra tetrad, if to the opposite pole, below the tetrad. All three dyads are seen to be homologues of the extra dyad first described and consequently of each other. Second spermatocytes derived from such complexes, containing, respectively, twelve, thirteen, and fourteen dyads, are illustrated in figures 37, 38, and 35 of plate 4.

The pairing of the triploid supernumeraries is somewhat analogous to the probable behavior of the three sex chromosomes in XX Y females in *Drosophila* (Bridges, '16). It seems that any two of these may synapse, but the two X's more often pair than an X with a Y.

D. Equational and reductional non-disjunction of the supernumeraries

Reductional non-disjunction of the members of the extra tetrad is illustrated in figure 136. Another cell where the two dyads of this tetrad were passing to the same pole was observed, but not drawn. The apparent failure of the two dyads of the extra tetrad to synapse in the complex shown in figure 85 might have resulted, too, in reductional non-disjunction. Since the publication of the results of Wenrich's ('16) valuable work on the spermatogenesis of *Phrynotettix* we cannot assume that the first or second maturation mitosis is the reduction division for a particular chromosome unless we know the history of the element in the early prophases of the first division or unless it happens to be a tetrad with unequal homologues. Hence, as each of the dyads resulting from the above three abnormal kinesis would divide in the second spermatocyte mitoses, the final outcome with respect to any one of the twelve spermatids might be either the equational or reductional nondisjunction of the supernumerary pair. Hence, also in the cell shown in figure 109, where one dyad of the extra tetrad has gone to one pole and the other dyad is dividing, we may have an instance of either reductional or equational non-disjunction. If, so far as the extra tetrad is concerned, this is a reducing mitosis, we have an instance of a unique kind of reductional non-disjunction. This is probably the case, for it is likely that the two members of the tetrad failed to synapse in this cell, otherwise one dyad would scarcely have lagged behind the other chromosome and divided. The results of this type of non-disjunction for the second spermatocyte complex formed at the upper pole are seen in figure 115. Here we have a second spermatocyte telophase with fourteen monads at one pole and thirteen at the other. The second spermatocyte complex derived from the chromosome group at the lower pole would be similar to those seen in figures 114 and 117.

In figure 108, where the two monads of an extra dyad have separated in a first spermatocyte division and gone to the same pole, we have the basis for equational non-disjunction in the next division. For these two monads would undoubtedly have segre-

gated freely and might have gone into the same spermatid. According to the counts given in table 3 (follicle A), another supernumerary dyad should have been found in the complex illustrated in figure 108. What became of this chromosome I do not know, but the complex cannot be accepted (in the absence of other unquestionable instances) as a case of variation within a first spermatocyte cyst. For the missing element, as sometimes happens, might have been pulled out of place by the microtome knife.

The instances of occasional aberrant behavior of the supernumeraries just given may explain the three exceptional complexes found in cyst 5 of 2511 (figs. 65 and 67) and the one in cyst 6 of 2525. For these second spermatocyte groups of eleven and fourteen chromosomes, in cysts where only twelve and thirteen groups should occur, would result from non-disjunction of the extra pair in the first maturation division.

The two unsynapsed dyads of the extra tetrad, too, shown in figures 85 and 136, as well as the pair in a similar complex which is not shown in the drawings, furnish added proof that the dyads of this tetrad are homologues of the unpaired supernumerary. They have the same size, form, fiber attachment, and position on the spindle.

E. Classes of spermatozoa in individuals with a varying number of supernumeraries

Since the supernumary within one individual (tables 1 and 2) may be either unpaired, paired, or present in triplicate in different follicles, six classes of spermatozoa may be formed in the testis of such an animal. If we denote the euchromosomes by 'e,' the accessory by 'x,' and the supernumeraries by 's,' these classes are:

11 e
 11 e + x
 11 e + s
 11 e + 2 s
 11 e + x + s
 11 e + x + 2 s

Should non-disjunction of a pair of supernumeraries occur in a first spermatocyte cell containing three extra dyads, these two additional classes are possible:

$$\begin{array}{l} 11 e + 3 s \\ 11 e + x + 3 s \end{array}$$

F. Summary of observations on the atypical complexes

Inconsistencies in the chromosome count within each of five individuals are due to the presence of supernumeraries. These elements are all apparently genetically related. The extra dyads are, in fact, homologues in size, form, and behavior. The supernumerary tetrads are formed by the synapsis of a pair of these homologous dyads.

Within the same individual, but in different follicles, the supernumerary may be absent in some complexes, present in an unpaired condition in some, paired in others, and present in triplicate in still others. But with the doubtful exception of one complex in twenty-two spermatogonial cysts, and one complex in fifty-two first spermatocyte cysts, the counts are consistent for the cyst. With these two exceptions the chromosome number seems to be consistent, too, for the follicle, prior to the second spermatocyte generation.⁵ When the extra element is unpaired in the first spermatocytes, it behaves precociously like the accessory in the late prophase, but shows no tendency to synapse with the latter. In the anaphase, it usually passes undivided to one pole, segregating freely with respect to the accessory. Occasionally the unpaired supernumerary divides in the first spermatocyte mitosis. When it does not divide in the first mitosis, it divides in the second; conversely, if it divides in the first it passes undivided into one daughter cell in the second mitosis. When the supernumerary element is paired in the first spermatocytes, it exhibits no marked differential behavior

⁵ It must be admitted, however, that the evidence for an unvarying chromosomal organization for the follicle is not as good as the evidence for such an organization in the cyst. For though counts were obtained in seventy-six follicles, in only fifteen of these were counts secured in two or more cysts.

in the late prophases and forms a tetrad like a typical euchromosome pair. This tetrad divides in metaphase like any other tetrad; the two halves divide again in the second mitosis, as in the case of other pairs. When the supernumerary is present in triplicate in a first spermatocyte cell, two of the extra dyads synapse to form a tetrad, the other behaves like a typical unpaired supernumerary and exhibits no tendency to form a hexad. Occasionally a supernumerary pair apparently fails to synapse. When this happens the free segregation of the two homologous dyads, and the fact that one of the latter may divide in the first mitosis, explains certain second spermatocyte counts, not in agreement with the rest of the cells in the cyst and follicle. With respect to the supernumeraries and the accessory, six (with possibly rarely eight) classes of spermatozoa may be formed in the testis of one individual.

DISCUSSION

A. Extra elements and numerical variations in the complex in other material

I have referred to variations in the chromosome number, due to the presence of one or more supernumeraries, among the individuals of a species reported in the Hemiptera by Wilson ('07 a, '07 b, '09 a, '09 b, '10), in the Diptera by Bridges ('13, '14, '16), in the Coleoptera by Stevens ('08, '12 b), in the Orthoptera by Stevens ('12 a), Carothers ('17), McClung ('17), Robertson ('17), and Wenrich ('17). These observers stress the constancy of the chromosome count within the individual. Wilson ('09 b, p. 185) alone mentions having observed in a few cells a numerical discrepancy. With this exception, no variation for the cells of the individual have been reported by the above authors. On the contrary, all, including Wilson, emphasize the fact that the zygotic complex apparently maintains itself from the time of fertilization to the first naturation division, and that there is no real numerical variation (with the above exception) in the first spermatocyte complexes of the individual.

Supernumeraries occur, too, in plant material. They have been reported for the *Oenotheras* by Gates and Thomas ('14),

Lutz ('12, '16, '17), and Hance ('18 a, '18 b). But no authentic instances of inconsistencies in the chromosome count within the germ tract of an individual, previous to the reduction divisions, seem to be recorded.

Holt ('17) has described multiple complexes in the degenerating gut cells during the pupal stage of *Culex pipiens*. Hance ('17 and '18 a) reported fragmentation of the somatic chromosomes in the pig and *Oenothera scintillans*, but states that the number of chromosomes in the germ cells is constant for each individual and species, and later observations by this author ('18 b), on better material, indicate that he might have been mistaken in regard to the somatic complexes of *scintillans*.

A well-known case of variations in the chromosome count of the individual is that of *Ascaris megalocephala* (Boveri, '99 and '04; Bonnevie, '01). In *Ascaris* the somatic chromosomes undergo segmentation; but here, as in the three species referred to in the preceding paragraph, no numerical variation is reported for the germ cells.

In this paper we are concerned only with variations in the germinal complex appearing within the individual. For this reason the foregoing and other apparent or real inconsistencies in the somatic chromosome count will not be discussed (Della Valle, '09; McClung, '17; Parmenter, '19).

Most workers on orthopteran material are familiar with the giant-cells (Hartman, '13) which sometimes occur among the spermatogonia and spermatocytes in the Acrididae. These contain usually double the number of chromosomes. Frequently they are clearly seen to be pathological and degenerating. It is evident that, although they may complete the process of transformation, they never produce fully functional spermatozoa, for, among the more than forty genera (McClung, '14, '17) of this family which have been studied, no grasshopper with more than two extra chromosomes has hitherto been reported.

Foot and Strobell ('12) state: "In *Euschistus variolarius* the number of independent chromosome fragments is most variable, a fact easily and definitely determined by the aid of the large number of photographs we have taken of complete groups of chromosomes in a single embryo.

"The same phenomenon was frequently found in the testis also and in some first spermatocyte mitoses, the isolated portion when present, behaving in every way like an independent chromosome exceptionally dividing in the equator of the spindle or passing undivided to one pole."

The above quotation suggests that there was some numerical variation, due to fragmentation or the presence or absence of supernumeraries within individuals of the species of Hemiptera referred to. But one is unable to form an opinion in regard to the real nature or extent of this variation from a perusal of the paper from which the above quotation is taken, or from a previous account of the same species by those authors (Foot and Strobell, '09). Nor do their photographs as published make the matter any clearer.

Payne ('14) reviews the work of earlier investigators (whose results were not in agreement) on *Forficula auricularia* and reports that the apparent numerical variations found in individuals of this species are due to the failure of certain spermatogonial chromosomes to pair at times during synapsis. He found that the elements which fail to conjugate may or may not divide in the first division. He was unable to determine if they divide in but one of the two maturation divisions as is typical for supernumeraries. In one individual variations were found in the spermatogonial complexes, but these were explained as possibly due to pathological conditions.

So with the exception of a few cells observed by Wilson ('09 b) and Payne ('14), there is no record of clear-cut numerical variations in the chromosome group within the germ cells of the individual, and even the above two exceptions to the rule of constancy in the chromosome count for the individual are not comparable to the wide variations just described for *Camnula*.

B. Nature of supernumeraries

If we accept the theory of the individuality, or the genetic continuity, of the chromosomes, it follows that unpaired extra elements are duplicates of chromosomes or parts of chromosomes of

the normal complex. It is conceivable and, indeed, probable that some odd supernumeraries are duplicates of only part of a member of a normal chromosome pair, while others are complete homologues of such a member. In the former case the extra elements must arise through breakage or fragmentation. Some evidence along this line is offered by 'deficiency' (Bridges, '17) and 'duplication' in *Drosophila* (Bridges, '18), although in the latter instance fragments do not become free supernumeraries. Further evidence is found in the unequal pairs reported by Carothers ('13, '17), Voinov ('14), Wenrich ('16, '17), and Robertson ('15). McClung ('17, pl. 7, fig. 59, and pp. 530 and 535) and Carothers ('17, pl. 11, figs. 38 *a*, 70 *d*, 70*e*, and pp. 465, 485, and 486) have directly observed the breakage of one member of a tetrad in certain first spermatocyte chromosomes of the Acrididae. I have seen one instance of the same thing in euchromosome no. 3. of *Camnula*.

Duplication of an entire member of a chromosome pair may result from the abnormal behavior of an element in a maturation mitosis. A chromosome pair may fail to synapse or there may be a lack of synchronism in the division of the tetrads. In the latter case we have the possibility of an element dividing precociously in the prophase, or, as suggested by Bridges ('16), division delayed until after the cell boundaries have started to form. Wilson ('09 b) observed the two members of the idiochromosome pair, presumably after a failure to conjugate, or a precocious division, passing to the same pole in three cells in *Metapodius*. Doctor Carothers has collected some valuable data, still unpublished, in regard to this matter. The work of Bridges ('13, '14, '16) has demonstrated that a supernumerary present in certain individuals in *Drosophila* is a duplicate of the Y element of the idiochromosome pair, and that it may be the result of: (1) Non-disjunction of the two X elements in the female at a maturation mitosis and subsequent fertilization of the XX egg by a Y sperm, or, (2) the formation of X Y sperm by non-disjunction of X and Y at the reductional division. Aberrant divisions in the first maturation mitosis have been observed, too, in the *Oenotheras* (Gates, '08 *a*, '08 *b*, '09, '11; Gates and Thomas, '14; Davis, '10, '11). I have found

a number of instances in *Camnula* where the two members of a pair had either failed to synapse or had divided precociously; one instance is shown in figure 10 where pair no. 10 exhibit this abnormal behavior.

Thus, there is evidence enough that unpaired supernumeraries may originate through the aberrant behavior at times of chromosomes in the maturation divisions.

I have found no records in the literature of observed abnormalities in the division of spermatogonial or oogonial chromosomes. I have observed none myself. In the metaphase seen in figure 133, plate 14, however, in the upper left quadrant, is a chromosome with a very marked constriction. In the same individual from which this drawing was made, another spermatogonial complex was found with two chromosomes having similar constrictions.⁶ It is conceivable that in anaphase a break might occur in one chromatid at the point of constriction, and one and one-half chromatids might pass to one pole, while the deficient chromatid passed to the other. There might thus arise an unpaired supernumerary and an unequal pair. Although no irregularities in the division of spermatogonial chromosomes that might give rise to a supernumerary, the duplicate of an entire member of the normal complex, have been reported, it is entirely reasonable to suppose that abnormal divisions sometimes occur. Extra elements may thus arise in the spermatogonia, as in the first spermatocytes, as the result of breakage or of premature or delayed divisions, followed by non-disjunction of the members of a chromosome pair.

Wilson identified ('07 b, '09 b) the from one to five supernumeraries occurring in certain individuals in *Metapodius* as extra idiochromosomes on account of similarities of size and form, behavior in the growth stages, and the fact that they typically conjugated with the idiochromosomes to form multiples of higher valence than tetrads. In one individual of *Metapodius femoratus* Wilson ('10) found that the unpaired supernumerary exhibited no precocity in the growth stage and united with the m-chro-

⁶ These constrictions in camnulan chromosomes do not appear to be constant for the race, or the individual, as in *Decticus* (Mohr, '19).

mosome pair, a member of which it resembled in size and form, to form a hexad in metaphase; he therefore identified it as an extra m-chromosome. An unpaired supernumerary in *Tettigidea* which behaved like the accessory in the growth stages and had a tendency to synapse with the latter in these stages, was identified by Robertson ('17) as a deficient extra accessory. Stevens ('08, '12 a, '12 b) in *Ceuthophilus* and *Diabrotica*, and Carothers ('17) in *Trimerotropis* and *Circotettix*, describe unpaired supernumeraries which simulate the behavior of the sex chromosomes in the growth stages, but show no tendency to couple with the latter. Stevens believed the supernumeraries she described were derivatives of the sex chromosomes, but Carothers is not inclined to identify the extra elements in *Trimerotropis* and *Circotettix* with the accessory.

In *Camnula* neither the unpaired supernumerary nor the extra tetrad have as yet been carefully studied in the early growth stages. In the later prophases of the first spermatocyte mitosis, the extra element, as we have seen, if unpaired, simulates the behavior of the accessory; if paired, it behaves like any euchromosome tetrad. In this instance precocity in the extra dyad seems to be due simply to its unbalanced condition, and does not necessarily indicate relationship with the accessory. The close resemblance which the extra tetrad sometimes bears to tetrad no. 3 has been pointed out. I believe by careful study of the earlier growth stages, and by breeding the animals, the derivation of the camnulan supernumerary can be demonstrated. Until this work has been attempted, it seems better not to speculate farther concerning the origin of the element in question.

Given a particular unpaired supernumerary, present in the germ cells of several individuals of a race occupying a limited area, it will happen sooner or later that two gametes, each containing this element, will conjugate. The resulting zygote will have naturally, a homologous pair of extra chromosomes. These may synapse in maturation in the typical manner to form tetrads as in *Camnula*, or may unite with the element from which they were originally derived to form multiples of a higher valence, as in *Metapodius* (Wilson, '09 b, '10).

Although the paired condition of the supernumerary may, and probably frequently does, originate in *Camnula* in the above fashion, through the process of fertilization, evidence has been presented that it does not always do so. A gamete containing two homologous extra elements is sometimes formed (see 'Classes of Spermatozoa'); both these elements may thus be inherited from one parent.

The triplicate condition of the supernumerary seen in figures 41, 52, 53, 54, 55, 56, and 64, if inherited from the parents, may be derived in *Camnula* in two ways. Obviously, where gametes, some with the extra element unpaired and some with it paired, are being formed in a population occupying a particular area, sooner or later a gamete of the first class should conjugate with one of the second class. The zygote would consequently have in its chromosome groups three homologous supernumeraries. The second method of origin of the triplicate condition is clearly of very rare occurrence, but may happen. It has already been pointed out, in connection with the tabulation of the classes of spermatozoa formed by the atypical individuals, that a gamete containing three extra homologues may be formed. It is thus possible for an animal to inherit all three supernumeraries from one parent. But it is not probable that any of the individuals considered in this paper inherited the supernumerary in a triploid condition.

C. Source of the variations in the complex within the individual

It is possible, I think, by an interpretation of the chromosome counts in individuals showing variations in number within the testis, to determine at what phase in the germ-cell cycle these variations arose.

Although mutations in the chromosome complex giving rise to supernumeraries have been observed to take place only in the maturation mitoses, we may suppose for the sake of argument that similar breakages in chromosomes and irregularities in division may occur anywhere along the germ line.

We do not know the early history of the germ cells in the *Acrididae*, but it is possible that all the germ cells in an individual

are the descendants of one primordial germ cell (Hegner, '14, '15). If an extra chromosome arises through breakage or some irregularity in division at the separation of the primordial germ cell from the soma, it may, of course, pass into either of the daughter cells. If it becomes a part of the first germ-cell complex, it should be present in all the germ cells formed in the testis. In this case the chromosome count would be constant for the gonad.

A supernumerary originating at the first division of the primordial germ cell should be found in half the germ cells of the mature gonad. If this extra element arose through the breakage of a member of the normal chromosome group, one of the daughter cells would have a deficient element in its complex. If non-disjunction of the two entire chromatids of a dyad were responsible for the appearance of the supernumerary, one daughter cell would lack altogether a member of some chromosome pair. As seems to be the case in certain instances of deficiency in *Drosophila* (Bridges, '17), a cell with a deficient chromosome pair might not be viable. When such a cell is not viable, the animal should develop a gonad composed of half the number of follicles. But the work of Carothers ('13, '17), Voinov ('14), Wenrich ('16, '17), and Robertson ('15) on deficient elements; the haploid condition found in parthenogenesis (Schleip, '08; Nachtsheim, '13); the 55-chromosome females in a 56-chromosome race of *Abraxas* (Doncaster, '14), the XO males in *Banasa* (Wilson, '07 b), and the XO non-disjunction males in *Drosophila* (Bridges, '16) demonstrate that a cell with a deficient chromosome pair may live. If it lived and fulfilled a normal rôle in development, half the complexes found in the germ cells of the gonad should contain an unequal pair or should lack one member of a pair.

The consequences of the mutation occurring at successively later stages in the germ line are obvious and are illustrated on the accompanying chart. If an abnormal division occurs, for instance, at the separation of a secondary from a primary spermatogonium, the chromosome counts will be constant for the cyst, but not for the follicle. If it occurs at the division of a spermatogonial cell, the counts will not even be constant for the cyst, and in the latter case, if complexes with an unequal pair or lacking

one member of a pair are not found within the cyst, when the cyst arrives at the first spermatocyte stage it should have less than the typical number of cells.

The variations in the second spermatocyte complexes have been shown to result from the normal free segregation of the accessory and the supernumeraries present in the first spermatocytes or from the occasional aberrant behavior of chromosomes in the first spermatocyte mitosis. Exclusive of the second spermatocytes, counts were obtained in some seventy-four cysts in the five animals with extra chromosomes. In seven of these cysts (early secondary spermatogonia) only one count was obtained in each cyst. In each of the other sixty-seven cysts from two to forty-three counts were made. In only two cells (table 3, A, 1; table 2, K, 13) were complexes found which might be considered exceptions to the statement that the numerical organization of the chromosome group is constant for the cyst. It was shown, too, in the analysis of the counts, that the fundamental numerical organization of the complex is constant for the follicle prior to the second spermatocyte generation.

The majority, if not all, variations in the chromosome group observed within the testis must have arisen, then, prior to the formation of the follicles. Nor, if there is only one primordial germ cell in *Camnula*, could they have arisen at the separation of this cell from the soma. They originated somewhere between the first cleavage of the primordial cell and the formation of the testis. Were it possible to secure accurate counts in all of the fifty or sixty follicles composing the camnulan testis, the particular cleavages at which the mutations took place might be determined. But this, unfortunately, cannot be done.

No complexes with an unequal pair or lacking one member of a normal pair, and no first spermatocyte cysts lacking a considerable proportion of the typical number of sixty-four cells were found in the atypical individuals. Hence it is probable that the numerical variations did not originate in the fragmentation or abnormal behavior of a member of the ordinary chromosome complex. They seem to be rather the result of the occasional aberrant behavior of the extra elements during mitosis

in the early germ cells. But one cannot be certain that degeneration on a significant scale did not occur among the cells of the germ tract in some of the atypical animals. For in some of these individuals the ultimate number of follicles in the gonad could not be determined on account of the immature age of the specimens or the method of sectioning.

Perhaps a word more should be said here about the degeneration of germ cells, for it may be asked if no degenerating cells were found in the testis of the atypical individuals. The answer is that they were, but they are no commoner in these individuals than in normal camnulan and in other orthopteran material. Every one who has studied such material is doubtless familiar with giant-cells containing multiple complexes and first spermatocyte cells in which all the chromosomes have failed to synapse. Such cells are frequently observed. They seem to be the source of the degeneration. I have been able to find no connection between the degenerating cells and the phenomena we have been discussing. Follicles containing extra elements are as frequently free from all traces of degeneration as normal follicles.

The diploid numbers in both individuals 950 and 2525 are twenty-three, twenty-four, and twenty-five. If we assume that each of these animals inherited a single unpaired supernumerary from one parent, the above three counts can be derived by one abnormal division of the extra element. A precocious or delayed division in a prefollicular but postprimordial germ cell, with the two halves of the extra element passing to the same pole, would explain these variations. For one of the daughter cells from this mitosis and its descendants would be without any extra chromosome, the other with its progeny would have a homologous pair of supernumeraries, while the remainder of the germ cells of this generation and their descendants would retain the inherited unpaired supernumerary. If this is the true explanation, the 24-chromosome class of cysts and follicles should be much larger than either of the other two. In individual 950 there are recorded three follicles of the twenty-three-chromosome class, six of the twenty-four-chromosome class, and six of the twenty-five class. In individual 2525 eleven follicles in the twenty-four-chromosome

class, three in the twenty-five class, and only one in the twenty-three class were found.

Individuals 980 and 2511 gave diploid counts ranging from twenty-three to twenty-six. (The fact that the twenty-four-chromosome class was not found in 2511 is hardly significant in such a small number of counts.) Assuming that these individuals each inherited the extra chromosome in duplicate, the numbers twenty-four and twenty-six can be accounted for by the non-disjunction of the two halves of one member of this pair at any of the earlier cleavages of the germ cells. To derive the twenty-three-chromosome complexes it would then be necessary to assume a recurrence of the non-disjunction process at a succeeding mitosis in the twenty-four-chromosome daughter cells.

If this is the way the variations in the complex originated the number of follicles belonging, respectively, in the twenty-three-, twenty-four-, twenty-five-, and twenty-six-chromosome classes should be in the proportion 1, 2, 9, 4. The chromosome counts recorded for these two individuals can also be derived from an inherited unpaired supernumerary by assuming two successive abnormal divisions of the extra element. In this case the expectation is that the class of follicles will give the proportion 4, 9, 2, 1. As a matter of fact, in individual 980 the ratio is 2, 2, 8, 1, and in 2511 it is 6, 0, 4, 2. But the number of follicles in which counts were obtained is relatively so small that not much significance can be attached to the ratios.

It is perhaps best not to speculate concerning conditions in individual 2526. Variation was discovered in only two prophase cysts, and in these the counts are uncertain. I am inclined to believe, however, that the mutation causing the variation in this animal must have occurred further along the germ tract than in the case of the other four aberrant individuals. For the count is constant for twenty follicles (all which contained metaphase figures).

Other interpretations of the phenomena recorded in this paper are, of course, possible. It is quite possible, for instance, that the numerical mutations that have been interpreted as occurring in four individuals prior to the formation of the testis may occur

in other individuals anywhere along the germ line. Even in the four individuals considered it is possible to account for the inconstant chromosome numbers by assuming that the variations arose during spermatogonial kinesis. But in the latter case it is necessary to assume also that the supernumerary frequently fails to divide normally within one individual. The data so far collected indicate that the element is quite regular in its behavior at mitosis. In only four first spermatocyte cells out of some hundreds examined was non-disjunction of the two halves of the supernumerary detected. The explanation of the varying counts that has been offered is believed to be the simplest one consistent with the facts, assuming for animals 950 and 2525 only one abnormal division of the supernumerary, and for 980 and 2511 but two such divisions. Nevertheless, I am well aware that further work on the members of this genus may invalidate some of the conclusions which have resulted from this preliminary study.

D. Accumulation or piling up of supernumeraries

As Carothers ('17) points out, in populations where unpaired extra chromosomes occur, on account of their free segregation in the maturation divisions, a piling up of these elements is to be expected. But since she never found more than two in any individual in *Trimerotropis* or *Circotettix*, she concludes that there must be some method of elimination.

Stevens ('12 b) found that the supernumeraries in *Diabrotica* might accumulate to a certain extent, for she observed five in one individual. Wilson ('09 b) also found as many as five in one individual in *Metapodius*, and these extra elements in *Metapodius* were seemingly homologues, although they segregated freely in the maturation division.

In *Drosophila* XXY individuals are viable, while XXX individuals are not (Bridges, '16).

I have examined some fifteen animals from the camnulan insular population of Puget Sound and have never observed more than three supernumeraries in a cell. Since some of the atypical ani-

mals were collected in 1909 and some in 1915, the extra elements or the tendencies to form them were probably transmitted through that interval of time. Extra chromosomes do not apparently accumulate in this material any more than in *Trimerotropis*. It may be that if more than three get into one gamete, some degenerate or are eliminated from the nucleus, or even the gamete may be non-viable as in the case of XXX zygotes in *Drosophila* ('16). But it is obvious since the supernumeraries in this material are genetically related and when paired synapse and segregate during maturation that, irrespective of the non-viability of gametes or the elimination of extra elements from the nucleus, a regulative process which insures disjunction and prevents piling up, to a great extent at least, is present.

E. The extra chromosome as a factor in heredity

When the entire chromosome complex is doubled, as in certain *Oenotheras* (Gates, '09, '11; Davis, '11), the somatic characters of the individual may be profoundly influenced by the doubling. In *Drosophila* we have a well-known instance of the effect of an extra chromosome on the soma (Bridges, '13, '14, '16). A single odd supernumerary may be one of the important factors in producing some *Oenothera* mutants (Lutz, '12, '16, '17; Gates and Thomas, '14; Hance, '18 a, '18 b).

The extra tetrad in *Camnula* is the only supernumerary pair, behaving at synapsis like a typical euchromosome pair, so far reported. Hence it is a matter of some importance to discover if there is any correlation between the distribution of this element and somatic characters. While the specimens from which came the material used in the present study are all preserved in this laboratory, it would be futile to attempt to work out the above correlation until a large number of individuals have been analyzed cytologically. This problem can be more satisfactorily attacked, too, with predigreed animals, and so may be laid over until it is possible to undertake some breeding work.

It seems likely that, in the germ tract of individuals in which it occurs, the *camnulan* extra chromosome may regularly suffer a

small per cent of non-disjunctions of its homologous strands. But this supernumerary has in all probability been inherited with the other members of the complex in one race of *Camnula* from 1909 to 1915. It must duplicate a whole or a part of one of the elements of the normal complex. Hence its influence on Mendelian ratios in any system of allelomorphs, which may be carried by it, is obvious, since certain loci may exist in a double, treble, quadruple, or quintuple condition. These various valencies of the loci in question may all occur, too, in different germ cells of the same individual. And if aberrant divisions of the extra element sometimes occur in early somatic mitoses, we have a mechanism for the production of mosaics.

SUMMARY AND CONCLUSION

In each of five individuals of the genus *Camnula* from islands in Puget Sound numerical variations occur in the chromosome group within the gonad. The normal or typical constituents of the complex are as constant in number in these animals as in some thirty others examined. The inconstant counts are due to the varying number of supernumeraries present in different cells within an individual. These extra elements are all homologous in size, form, and behavior, and are apparently genetically related. Within one individual the supernumerary may be absent in some complexes, unpaired in some, paired in others, and present in triplicate in still others. If unpaired, the extra element usually passes undivided into one of the daughter cells at the first maturation mitosis, segregating freely with respect to the accessory, and dividing like the accessory in the second mitosis. Exceptionally the extra dyad may divide in the first mitosis; in this case the extra monads pass undivided into one or the other spermatid at the second spermatocyte mitosis. If the two members of the supernumerary pair are present, they synapse like any euchromosome pair in the first spermatocyte generation to form a typical tetrad, which behaves in all respects like any of the other tetrads. This is in contrast to the same element when unpaired, which then is precocious, like the accessory, in

the post spireme stages. If the supernumerary is present in triplicate, two of the homologues synapse at the first mitosis, while the other remains free and segregates independently.

Although, as a result of the inconstant valence of the supernumerary, the numerical organization of the complex varies in the above five animals within the gonad, the count, with two doubtful exceptions (one a spermatogonial metaphase, the other a first spermatocyte telophase), is consistent for the cyst. The chromosome number is probably constant also for the follicle until after the first spermatocyte mitosis.

Two instances of the reductional non-disjunction of the dyads of the supernumerary tetrad in the first maturation mitosis were observed. A case of either maturational equational non-disjunction or a peculiar type of reductional non-disjunction of the chromatids of the extra tetrad was found. The basis for the equational non-disjunction of the two monads of a supernumerary dyad was seen in one cell, and the basis for the non-disjunction of the members of the extra tetrad in another cell.

The varying combinations of the supernumerary found in individual 950 and 2525 prior to the second spermatocyte generation, must be due to equational non-disjunction, and can be derived from a single non-disjunction of the two halves of an unpaired inherited extra chromosome at a prespermatogonial division. In individuals 980 and 2511 it is necessary to assume two successive equational non-disjunctions of inherited supernumeraries.

A single aberrant individual, such as 980 or 2511, produces, with respect to the accessory and the supernumeraries, six classes of spermatozoa and may occasionally form two additional classes.

The origin of the unpaired extra chromosome is unknown. If it is a duplicate of an entire member of the normal complex, that element is euchromosome no. 3, which it resembles in size and form.

An element such as the camnulan supernumerary, which has apparently been transmitted in this race for at least six years, may obviously have a significant influence on Mendelian ratios. This chromosome too, may furnish a mechanism for the production of mosaics.

Although the camnulan supernumerary may be present in varying combinations in different follicles of a gonad, it seems nevertheless to reproduce itself regularly at mitosis and to maintain its identity throughout the spermatogonial and spermatocyte generations. Since we can observe, too, most of the steps in the formation of some of its combinations, from the first spermatocyte metaphase to the second spermatocyte telophase, it offers evidence for, rather than against, the doctrine of the genetic continuity of the chromosomes.

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PLATES

The following are drawn at a magnification of approximately 3100 diameters: Figures 40, 41, 42, 62, 63, 64, 96, 97, 98, 99, 101, 131, 132, and 133. All other drawings are made at a magnification of 2800 diameters. The figures have been reduced one-third in reproduction.

All the drawings on any one plate are from a single individual; the number of the individual is placed at the top of the plate in the center. Each drawing has three identification marks, thus $\frac{1}{B3}$. The number above (1) is the serial number of the drawing. The letter (B) indicates which follicle of the particular testis the complex is from. The number (3) to the right of the letter locates the cyst. In the example given, figure 1 is a drawing of a chromosome complex in cyst 3, follicle B, of individual 950. Hence in the plates it is an easy matter to identify the complexes from the same follicle or cyst.

In most first spermatocyte complexes drawn the chromosomes have been serially arranged according to size. In such drawings the elements from a single complex are placed in a horizontal row, while the homologous chromosomes from different complexes appear in vertical columns. The number of the drawings, with the follicle and cyst from which they are taken, are placed at the left and the numbers of the chromosomes at the top. The serial arrangement is only approximate for some elements. The smallest two and the largest two are clearly differentiated from all the rest. Chromosomes *S*, 3 and 4 can be recognized by their size and certain peculiarities in form and behavior. But it is not possible to identify accurately elements 5, 6, 7, 8, 9, 10.

No. 4 is the accessory, which, as indicated, usually does not have as smooth an outline as the others. The accessory is labeled *X* in some drawings. In drawings in which the first spermatocyte elements are serially arranged, the pole toward which the accessory is passing is represented by the top of the page. The supernumerary, whether single, paired or triploid, is indicated by *S*; the chromatoid body and chromatoid granules by *C*.

PLATE 2

EXPLANATION OF FIGURES

Complexes from individual 954.1 (a normal animal)

- 11 to 14 Lateral views of first spermatocyte complexes.
- 15 and 17 Polar views of first spermatocyte metaphases.
- 16, 19, and 20 Second spermatocyte metaphases.
- 18 Polar view of a spermatogonial metaphase.

2511

	12	11	10	9	8	7	6	5	4	3	2	1
52 711	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
53 711	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
54 711	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
55 711	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
56 711	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
57 E7	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
58 E7	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
59 E7	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
60 E7	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂
61 E7	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂	✂

PLATE 7

EXPLANATION OF FIGURES

Complexes from individual 2511 (atypical)

- 62 Spermatogonial metaphase of twenty-three dyads.
- 63 Spermatogonial complex of twenty-five dyads.
- 64 Spermatogonial metaphase of twenty-six dyads.
- 65 to 68 Polar views of second spermatocyte metaphases with eleven, twelve, fourteen, and thirteen dyads, respectively.
- 69 to 71 Lateral views of second spermatocyte metaphases, each with thirteen dyads.

2511

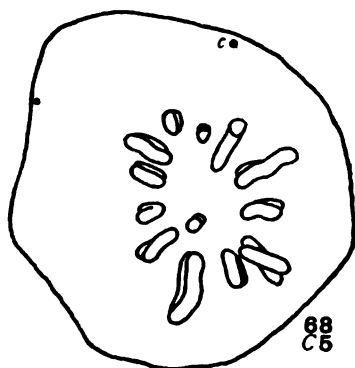
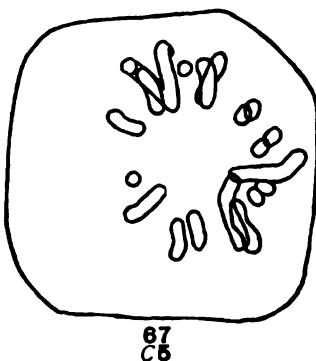
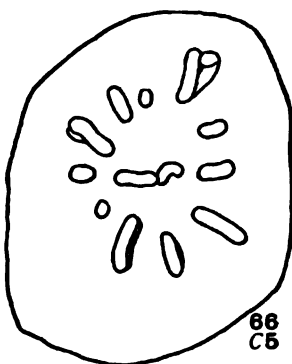
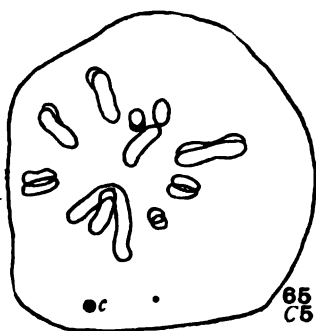
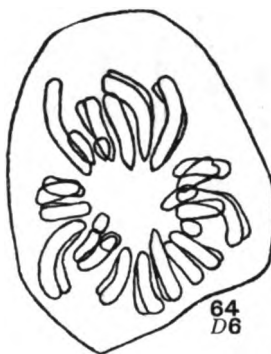
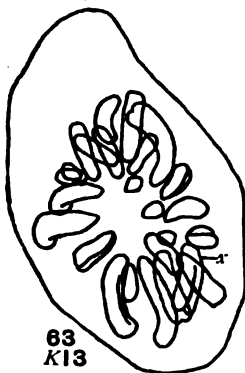
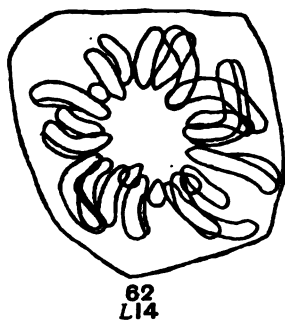


PLATE 8

EXPLANATION OF FIGURES

Lateral views of first spermatocyte complexes from atypical individual 2525

72 and 73 Complexes with the extra tetrad (*S*).

74 to 81 Complexes with the extra dyad (*S*).

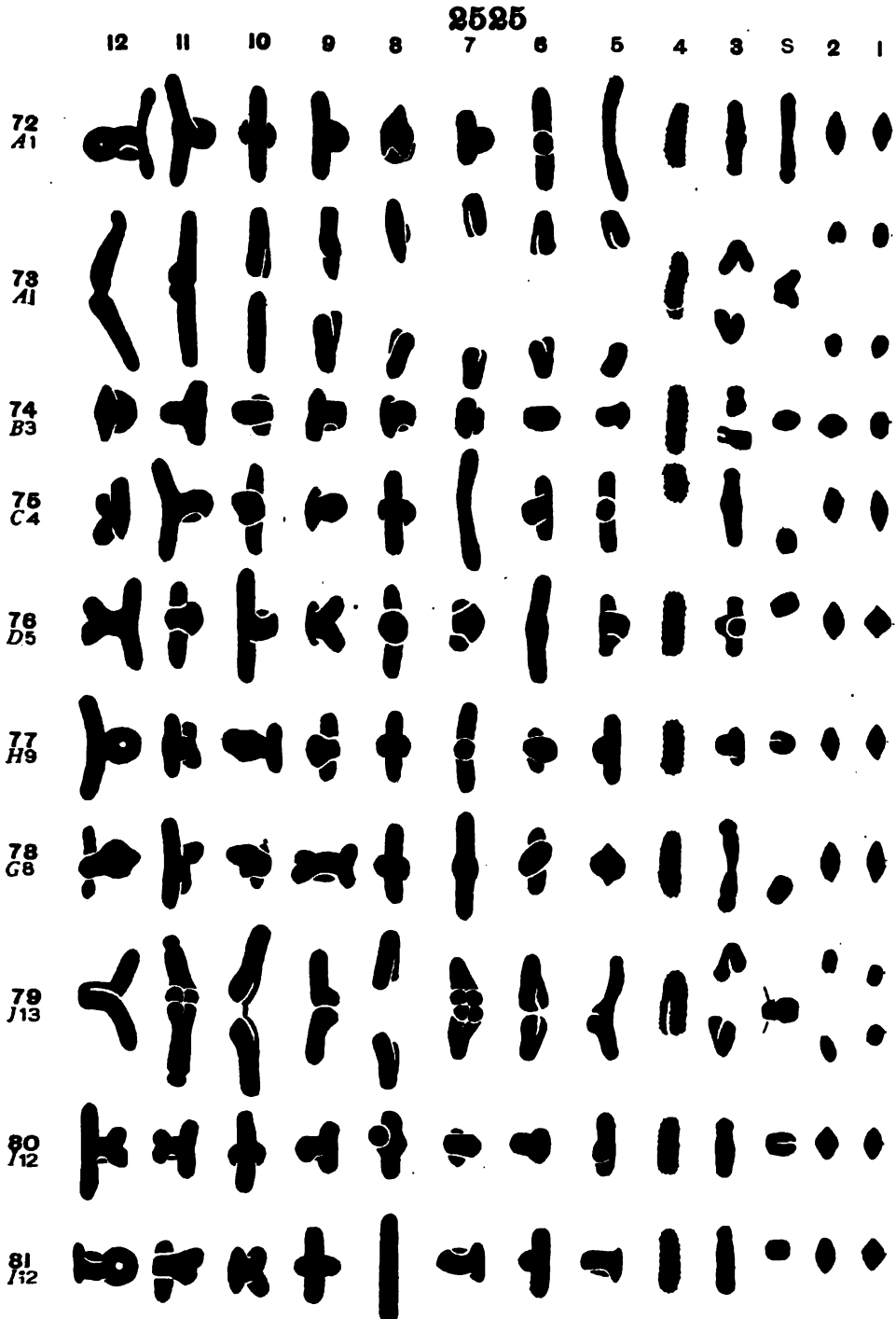


PLATE 9

EXPLANATION OF FIGURES

Lateral views of first spermatocyte metaphases from individual 2525
(aberrant)

82 to 84 and 86 Complexes containing the supernumerary tetrad (*S*).

85 Complex in which the two dyads (*S*) of the extra tetrad have failed to synapse or have divided prematurely in early prophase.

87 to 90 Complexes with the normal number of chromosomes.

91 and 92 Complexes with the extra dyad (unpaired supernumerary).

2525

	12	11	10	9	8	7	6	5	4	3	S	2	1
82 K14													
83 K14													
84 K14													
85 K14													
86 K14													
87 L16													
88 L16													
89 L16													
90 L16													
91 M17													
92 N18													

PLATE 10

EXPLANATION OF FIGURES

Complexes from atypical individual 2525

93 Lateral view of first spermatocyte spindle showing complete complex of eleven tetrads, plus the accessory (*x*) plus the extra dyad (*S*).

94 Oblique view of first spermatocyte complex with the extra tetrad (*S*).

95 Polar view of normal first spermatocyte complex without any extra elements.

96, 98, 99, and 101 Spermatogonial metaphases of twenty-four dyads.

97 Spermatogonial metaphase of twenty-five dyads.

100 Second spermatocyte metaphase (polar view) of twelve dyads.

102 Lateral view of second spermatocyte anaphase with twelve monads passing to each pole.

103 Polar view of second spermatocyte metaphase of thirteen dyads.

104 Lateral view of second spermatocyte anaphase with thirteen monads passing to each pole.

(Note:—The complexes shown in figures 102 and 104 were each in two sections and the chromosomes are drawn displaced somewhat from their natural positions in the cell.)

2525

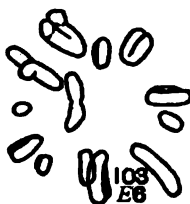
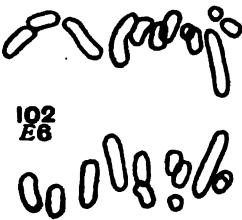
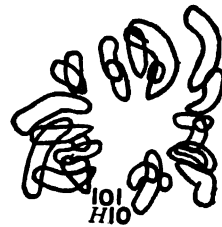
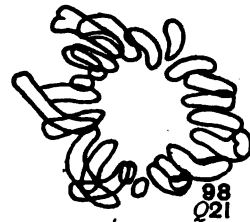
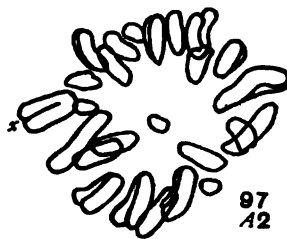
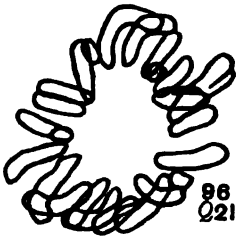
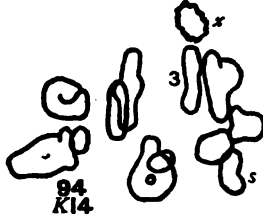
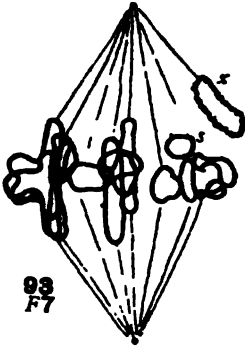


PLATE 11

EXPLANATION OF FIGURES

First spermatocyte complexes from atypical individual 2525

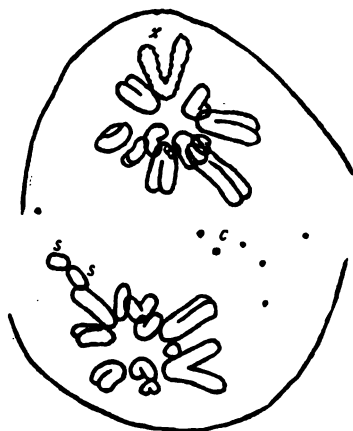
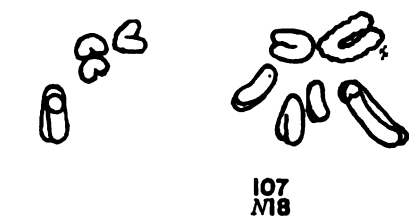
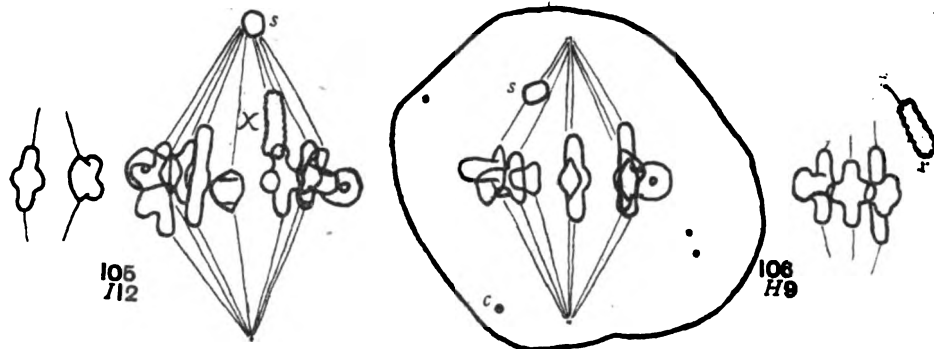
105 to 107 illustrate the free segregation of the unpaired supernumerary with respect to the accessory. In the lateral views of metaphases seen in figures 105 and 106 the extra dyad (unpaired supernumerary) is passing to the same pole with the accessory. In figure 107, a lateral view of a telophase, it has gone to the opposite pole.

108 illustrates what might have become a case of equational non-disjunction (Bridges, '16) in the second mitosis. Two monads (*S*) of the extra dyad have separated during the first division, but have gone to the same pole; in the second mitosis they would probably segregate freely and hence might pass into the same spermatid. This telophase, according to other counts in the cyst and follicle, should have an extra dyad at the other pole. No trace of it could be found. It may have been dragged out of place by the microtome knife. It is one of the two exceptions to the rule of numerical constancy within the cyst.

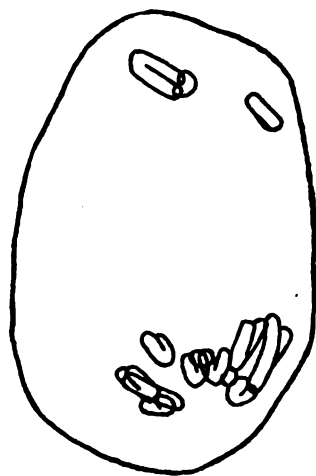
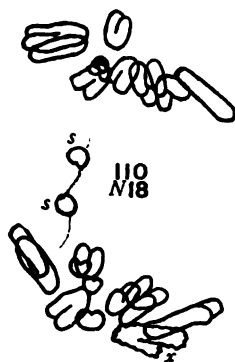
109 illustrates either equational non-disjunction or a peculiar type of reductional non-disjunction. The two dyads of the extra tetrad failed to synapse or divided precociously. One passed to the upper pole. The two monads (*S*) of the other have separated and are passing to opposite poles.

110 illustrates the occasional division of the unpaired supernumerary (*S*) in the first mitosis.

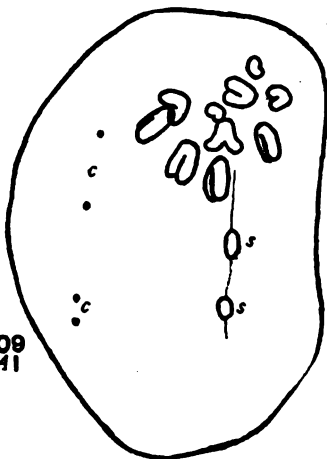
2525



108
A1



109
A1



449

PLATE 12

EXPLANATION OF FIGURES

Complexes from atypical individual 2525

111, like figure 110 of plate 11, is an anaphase illustrating the occasional division of the unpaired supernumerary in the first mitosis. Note that when this occurs the supernumerary lags behind the other chromosomes somewhat in division.

112 A late first spermatocyte anaphase with the extra tetrad (*S*) not yet divided.

113 Second spermatocyte telophase with twelve monads at each pole.

114 and 117 Second spermatocyte metaphase spindles, showing in each case the entire complex of eleven dyads plus one monad. The monads (*S*) have resulted from the division of the extra dyad in the first mitosis and are here seen passing to the poles without, of course, dividing.

115 A second spermatocyte telophase with fourteen monads at the upper pole and thirteen at the lower. This complex was derived from a group like that seen at the upper pole in figure 109 and is based on the same type of non-disjunction. During anaphase the exceptional monad passes to one pole, and thus we get the two groups of thirteen and fourteen monads.

116 A second spermatocyte telophase with thirteen monads at each pole.

118 and 119 illustrate the precocious behavior of the unpaired supernumerary (*S*) in late prophase. This element, like the accessory, condenses more rapidly than the other chromosomes and thus appears smoother and less granular at this stage.

2525

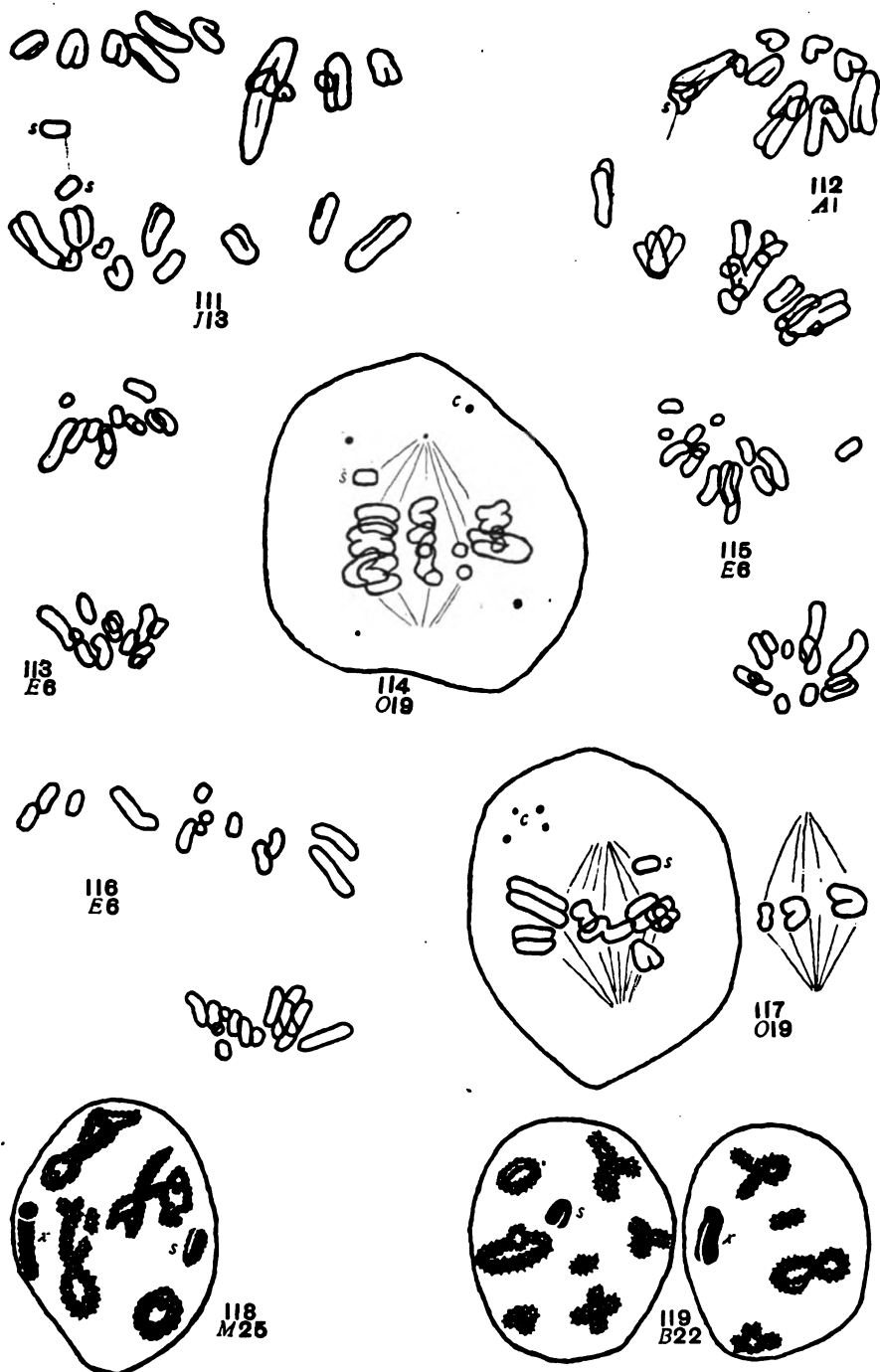


PLATE 13

EXPLANATION OF FIGURES

First spermatocyte complexes from atypical individual 2526

120 to 128 Lateral views.

129 and 130 Polar views.

There is a supernumerary pair (*S*) present in all spermatogonial and first spermatocyte cells containing metaphase figures in this individual. But the chromosome count is not constant, for two prophase cysts containing the supernumerary in an unpaired condition were found.

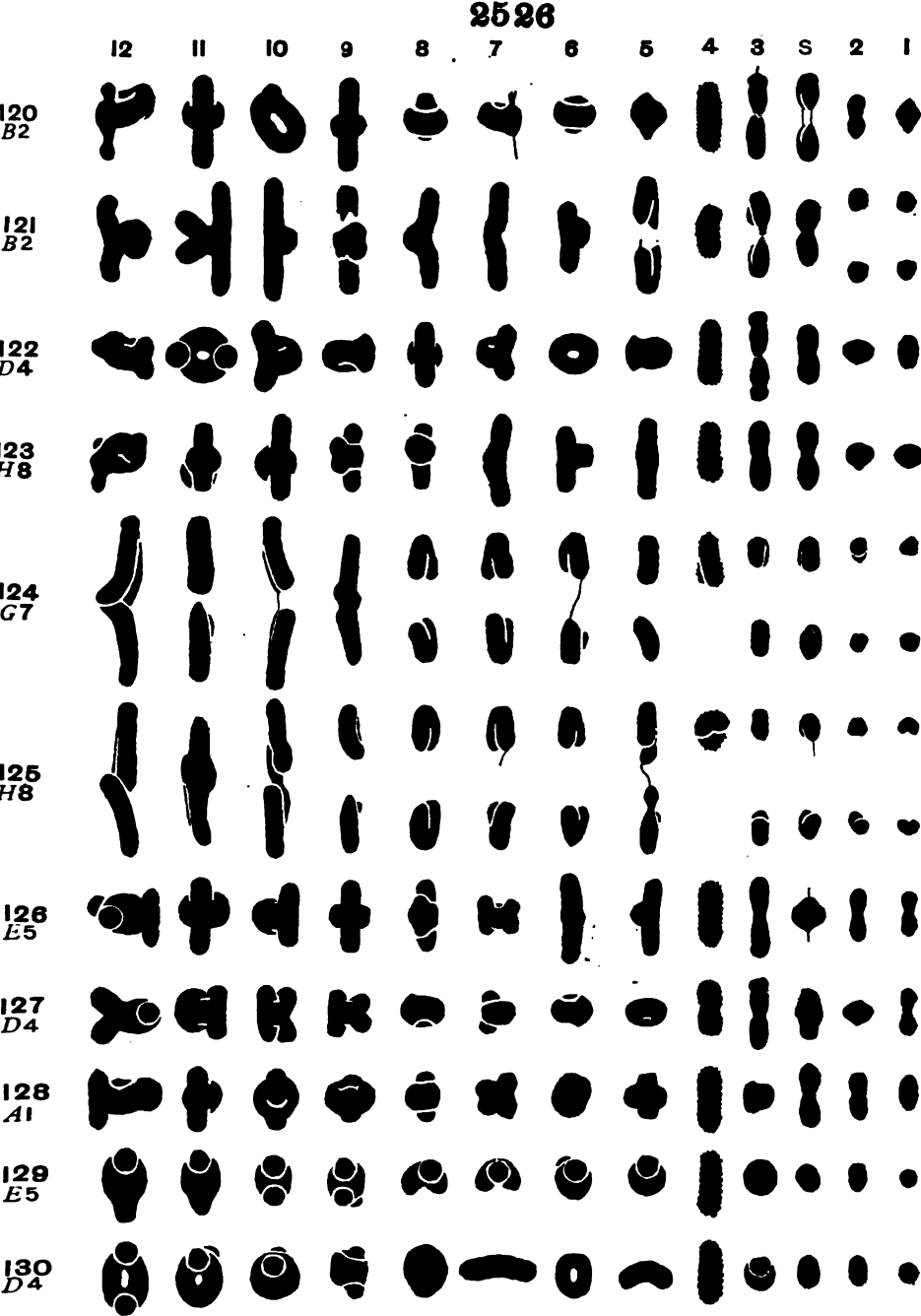


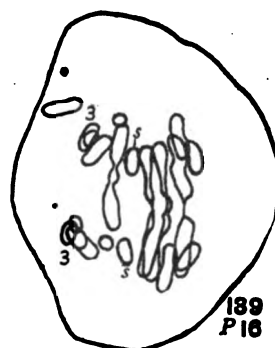
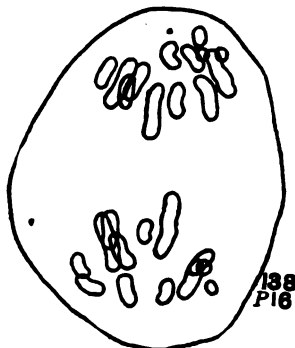
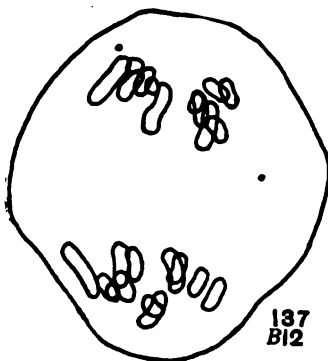
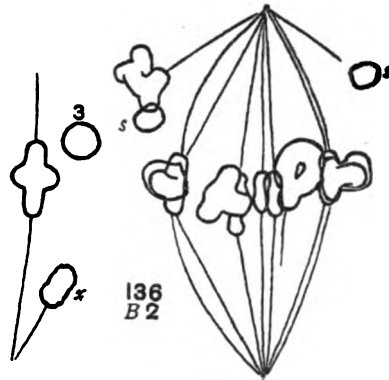
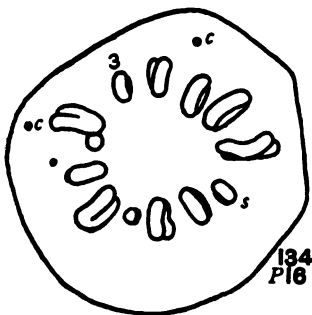
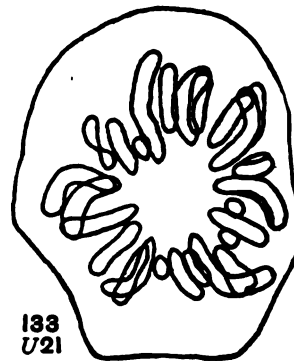
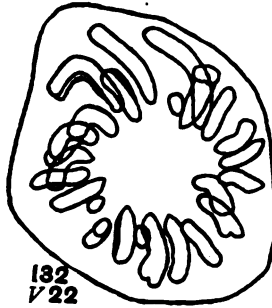
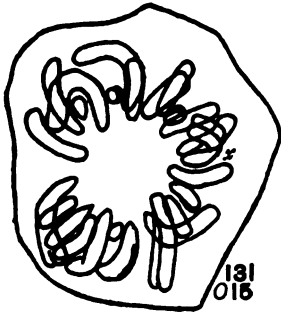
PLATE 14

EXPLANATION OF FIGURES

Complexes from atypical individual 2526

- 131 to 133 Polar views of spermatogonial metaphases of twenty-five dyads.
- 134 Polar view of second spermatocyte metaphase of thirteen dyads.
- 135 Euchromosome no. 3 and the extra tetrad (*S*) from the same cell. Note the similarity of form. The supernumerary is somewhat foreshortened.
- 136 Lateral view of a first spermatocyte spindle showing entire complex and the reductional non-disjunction of the two dyads of the extra tetrad (*S*).
- 137 and 139 Second spermatocyte anaphases with twelve monads passing to each pole. *S*, the extra monads.
- 138 Second spermatocyte anaphase with thirteen monads passing to each pole.

2526



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DECEMBER, 1920

Resumen por los autores, W. R. Coe y S. C. Ball.
Osborn Zoological Laboratory, Yale Universit.

El nemertiano pelágico *Nectonemertes*.

Un detenido estudio de preparaciones aclaradas y cortes seriados de ejemplares pertenecientes al nemertiano pelágico *Nectonemertes mirabilis*, descrito por Verrill bajo este nombre hace muchos años, demuestra que esta especie presenta dimorfismo sexual. Los machos que han alcanzado la madurez sexual están provistos de un par de tentáculos musculares, cuya estructura describen detenidamente los autores en el texto.

Los espermarios, situados en los bordes laterales de la cabeza, están provistos de paredes musculares resistentes y cada uno de ellos comunica con una gran vesícula seminal. Las hembras, desprovistas de tentáculos, han sido consideradas como especies pertenecientes a otro género, el *Hyalonemertes*. Los autores describen con detalle cada uno de los órganos del cuerpo, indicando también la adaptación de estos gusanos a la vida batipelágica.

Translation by José F. Nonidez
Cornell Medical College, New York

THE PELAGIC NEMERTEAN NECTONEMERTES

WESLEY R. COE AND STANLEY C. BALL

Osborn Zoological Laboratory, Yale University

FIVE PLATES (SEVENTEEN FIGURES)

More than twenty-five years ago Verrill ('92) described a species of nemertean which differed from all other known flatworms in the possession of a pair of long muscular appendages, cirri, or tentacles, attached to the sides of the body immediately back of the head (fig. 1). This was made the type of a new family, Nectonemertidae, and was named *Nectonemertes mirabilis*. Since the publication of Verrill's original description of the type species a number of other species and genera of closely related pelagic nemerteans from the deep oceans in widely separated parts of the world have been collected. Opportunity has now been afforded us for a detailed anatomical study of the specimens described externally by Verrill, whereby it is possible to supplement his descriptions by a more complete account of the internal structures and so add somewhat to the available knowledge of nemertean organization and relationships.

Verrill's description and figure indicate a type of structure well adapted to pelagic life. The specimens were collected in the North Atlantic Ocean in regions where the water was from 600 to 1700 fathoms in depth. Verrill also describes ('92) two other specimens from about the same region which closely resembled *Nectonemertes* in form, size, general appearance, and structure, but which were without the lateral appendages. These he made the type of a new genus and species, *Hyalonemertes atlantica*, but, as will be explained below, it now seems evident that they represent the females of the sexually dimorphic *Nectonemertes mirabilis*.

Besides the five specimens studied by Verrill, one additional excellently preserved specimen, which was collected by the U. S.

Fish Commission from the same region of the Atlantic, has recently been sent us.

Another individual, collected in the eastern Atlantic off the coast of the Azores, was described superficially by Joubin ('04) under the new name *N. grimaldii*, but as no detailed anatomical study was made, it is still uncertain whether or not it is specifically distinct from *N. mirabilis*.

Still another species, *N. pelagica*, occurs off the coast of California, and has been very fully described and excellently illustrated by Cravens and Heath ('06). Three specimens of this species were taken by fishermen in Monterey Bay and two others collected in a trawl off the southern coast of California. In these localities the water is from 400 to 2200 fathoms in depth. The color is described as brilliant scarlet. All were males.

Joubin ('06) later described from a superficial study, but without the aid of sections, two additional specimens of *Nectonemertes*, each of which he considers the type of a new species. These are: *N. chavesi*, with rudimentary tentacles represented merely by a pair of blunt lateral lobes to the body walls, and *N. lobata*, with a slender transparent filament at the end of the tentacles. Of these the former has the cephalic gonads characteristic of the males, while the gonads of the last named were not noticed, but may have been represented by "petites papilles très courtes font saillie autour de l'orifice buccal" (p. 20). Hence this specimen was also probably a male.

Joubin also described six new species of deep-sea nemerteans taken from the same general regions in the eastern Atlantic and represented by eight specimens without tentacles. All of these he refers with more or less hesitation to the genus *Planktonemertes*. One of these, *P. rhomboidalis*, has cephalic gonads, and may be definitely considered as belonging to that genus. Of the five remaining species, two, *P. grimaldii* (with two specimens) and *P. zonata* (also with two specimens) have the gonads distributed along the sides of the body, indicating that they are females. The descriptions of *P. alberti* and *P. sargassicola*, each described from a single specimen, have no mention of gonads, while the only specimen of the sixth species, *P. elongata*, is believed by Joubin to be immature.

The evidence points strongly to the probability that one or more of these supposed species of *Planktonemertes* will eventually prove to be the females of the species of *Nectonemertes* which are found in the same regions of the Atlantic; that is, between the Sargasso Sea and the Azores.

Bürger ('09) has described the anatomy of one male specimen under the name *N. mirabilis* and a female which he identified as *Hyalonemertes atlantica*. Brinkmann ('17 a) shows the former to belong to *N. primitiva* and the latter to *N. minima*.

Still another species of *Nectonemertes*, *N. japonica*, has been described by Miss Foshay ('12) from six specimens collected near Misaki, Japan. All of these have well-developed tentacles and all are males.

This makes a total of more than twenty specimens of *Nectonemertes* known up to that time in which the sex is known and all are males. In *Hyalonemertes*, on the other hand, the few specimens studied are without cephalic gonads and are either known to be females or are presumably of that sex. But as the only apparent differences between *Nectonemertes* and *Hyalonemertes* are the presence or absence of tentacles and the differences in the sexual organs, and as the tentacles develop in the males only with the sexual maturity of the animals, the conclusion appears obvious that *Nectonemertes* is the sexually differentiated male of the various species, while *Hyalonemertes* and some of the specimens described as *Planktonemertes* represent the corresponding females. The species must all bear the generic name *Nectonemertes*, since this has priority.¹

This view was first advanced by Brinkmann ('12, '17)² who

¹ Positive confirmation of the sexual dimorphism of this form has more recently been furnished by Brinkmann ('17, '17a), who has secured for study no less than 116 specimens, including 32 males with tentacles, 6 young males, 63 females, and 15 young, sexually undifferentiated individuals.

² The males form a regularly graded series in which the growth of the tentacles is perfectly correlated with the state of development of the gonads. In this series the tentacles range in size from the merest indications of lateral outgrowths of the body walls to the fully developed appendages two or three times as long as the width of the head. The females, on the other hand, show no trace of these appendages at any stage of life. Furthermore, a similar sexual dimorphism is shown by Brinkmann ('17) to exist in two other species of the genus. In *Balaenanemertes*, however, a pair of very short tentacles is found in both sexes.

states that he has been able to prove from a large series of specimens that sexual dimorphism actually occurs and that the lateral tentacles develop in Nectonemertes only after the animal reaches sexual maturity. Somewhat more recently, Brinkmann ('16) has found in a closely related species, *N. minima* Brink, a parallel case in which the three males which he had for study showed the tentacles in various degrees of development, while the single female specimen available for study showed no traces of such appendages. He looks upon these peculiar appendages as having become specialized to serve as grasping organs by which the male is able to cling to the female during the act of insemination. There seems little doubt that this interpretation is correct. Living as the worms do in the vast areas of the deep sea and not limited, as are so many other forms, to a very restricted range of depth either at the surface or on the ocean's floor, these nemerteans doubtless ordinarily live as widely separated individuals. This is evidenced by the rarity with which they are taken, even when many hauls of the net are made in the same locality.

With this separation of the individuals the ordinary methods of fertilization which obtain in littoral nemerteans, namely, the free discharge of eggs and sperm into the water at the place where the two sexes are living, would be extremely hazardous. To insure fertilization in this way there is required proximity of the sexes in restricted areas. Hence the advantage of making insemination reasonably certain by free motility of the animals and the provision for their remaining in contact for a period sufficiently extended to allow for the transfer of the sperm. Both these advantages, or necessities, are adequately provided for by the muscular tentacular appendages of the male in Nectonemertes.

Correlated with these adaptations is a peculiar modification of the spermaries, which are provided with strong muscular walls, as described below, for the forcible ejection of the sperm. Correlated also is the great reduction in the number of eggs produced by the female. Here, as in other bathypelagic species, not only is the number of ovaries greatly reduced, as compared with other nemerteans, but each ovary brings to maturity only one or two ova. All the rest of the numerous primitive ova become reduced

to nurse cells and are eventually absorbed by the one or two which mature. But these latter far exceed in size the eggs of the littoral species.

There is thus a remarkable provision for insuring the development and fertilization of relatively few eggs provided with a large amount of food material. The studies of Brinkmann ('17, '17a) show that the period of reproduction may be very long, for there is no evidence of any influence of the seasons at the great depths at which these worms live.

EXTERNAL ANATOMY OF *N. MIRABILIS*

Turning now to the anatomical peculiarities of *Nectonemertes*, we find our material is such that we are enabled to supplement Bürger's account ('09) in certain details, as well as the very excellent descriptions which Cravens and Heath ('06) have published for *N. pelagica*, and Brinkmann ('17, '17a) for *N. mirabilis*, *N. minima* and *N. primitiva*.

As shown in figures 1, 4, and 5, the shape of the long oval, flattened body is well adapted for a free-swimming existence in the intermediate depths of the ocean. In all cases where the precise locality is known the various species inhabit regions having a great depth of water, usually of a thousand meters or more. In most instances they have been taken in an open net or occasionally caught on fishing lines, in which cases there was no proof whether they came from the bottom or the surface or from any intermediate depth. The recent reports of Brinkmann ('17, '17a), however, show conclusively that the species lives only at such great depths as have a temperature of 6° C. or less and a salinity not exceeding 35 per cent. In the North Atlantic Ocean these conditions rarely occur at less than 500 fathoms, and this is about the upper limit of the species.

Size and shape. The body, as was well described by Verrill ('92), is elongated elliptical, much flattened; with a pair of broad, thin lateral extensions of the body walls in a region from two-thirds to four-fifths the distance from head to posterior end of body, forming a pair of horizontal fins. The body is con-

stricted laterally behind these fins and then broadened at the posterior end to form a well-developed caudal fin (fig. 1). Just behind the head is a short neck region which gives rise to a pair of slender appendages, cirri, or tentacles, in the males only. The length of the tentacles increases with sexual maturity; when well developed becoming more than twice as long as the width of the body. The shape is well adapted for sluggish swimming in the depths of the ocean. The specimens available for study varied from 30 to 38 mm. in length and from 7 to 9 mm. in width, with a thickness of about 2 mm. after many years' preservation in alcohol. Verrill ('92) however, states that the largest specimen when first seen by him was about $2\frac{1}{2}$ inches long and $\frac{1}{2}$ inch wide.

The fact that preservation in alcohol has reduced the body to about one-fourth its original bulk indicates the extremely gelatinous consistency of the living animal. The females described as *Hyalonemertes atlantica* by Verrill ('92) were from 20 to 38 mm. long and from $3\frac{1}{2}$ to 11 mm. wide.

Color. The living animals are described by Brinkmann ('17, '17a) as varying from red to orange red; the color being due to the contents of the intestinal cells. The margins of body are, consequently, colorless. This agrees with the color of one of Verrill's specimens, which was seen by him shortly after preservation, and was salmon or pale orange colored. *N. pelagica* is, according to Cravens and Heath ('06), bright scarlet. All observers mention the great translucency of the body in this and related forms.

Habitat

The seven specimens collected by the U. S. Fish Commission came from the North Atlantic Ocean at four stations between about 37° and 42° N. Lat., and 50° to 73° W. Long., the depth of water in this region being from 600 to 1700 fathoms. The recent studies of Brinkmann ('17, '17a) show that the worms are strictly bathypelagic, and that the species occurs throughout the entire width of the Atlantic Ocean between 35° and 64° N. Lat. at a depth of 500 fathoms or more.

INTERNAL ANATOMY

Body walls

Integument. In all the specimens the epithelial covering of the body was almost entirely dislodged, leaving the ragged surface of the basement layer exposed. This loss of the body epithelium usually occurs in bathypelagic nemerteans during their capture. This is probably due in part to the change in pressure of the water while they are being brought from a depth of hundreds of fathoms to the surface and also in part to the great delicacy of the epithelial covering, making it easily removed in handling. What epithelium remains consists of a delicate layer of columnar cells, as described and figured by Cravens and Heath ('06) for *N. pelagica*.

Basement layer. In distinction to the epithelium, the basement layer (figs. 6, 16, *bm*) is firm, but extremely thin as compared with that of littoral nemerteans. Its surface is thrown into irregular folds and corrugations for the attachment of the epithelium.

Musculature. The body musculature is very much reduced, particularly along the lateral margins. Only in the region of the tentacles and in the caudal fin are the muscles on the sides of the body well developed. Elsewhere the circular layer consists of a thin sheet of tissue (figs. 6, 15, 16) directly beneath the basement layer. The longitudinal muscles extend as broad sheets along the dorsal and ventral sides of the body, but almost disappear laterally (fig. 10).

The longitudinal muscles are also much thinner along both median lines, but particularly on the dorsal side, directly above the proboscis sheath. In the caudal fin, on the contrary, they are well developed on all sides (figs. 8, 9).

At intervals throughout the length of the body bundles of dorsoventral fibers connect the muscular layers of dorsal and ventral surfaces directly through the body parenchyma, and between the intestinal diverticula. These dorsoventral bundles are likewise particularly highly developed in the caudal fin and in the tentacles (figs. 8, 9, 12, 13), as well as in the keel-like horizontal fins

(fig. 4). The nuclei of these muscles are often conspicuous about the middle of their length (figs. 8, 9). The dorsoventral muscles closely invest the proboscis sheath and anchor it in place in the delicate parenchyma. Many of the bundles pass out radially from this organ into the muscular layers of the body walls.

The character of the musculature clearly indicates that the animal is a sluggish swimmer, depending for locomotion largely on the movements of the caudal fin, and that it has little capacity for changing the shape of its body or exhibiting the rapid undulations characteristic of littoral species. The special musculature of the tentacles and the function of these organs will be described below.

Parenchyma. As is other pelagic nemerteans, the abundance of the gelatinous parenchyma which surrounds the internal organs (figs. 6, 8, 14) and separates them widely gives the animal its translucent appearance and adapts it to its pelagic existence.

THE TENTACLES, OR NUCHAL CIRRI

The most remarkable feature of this genus, other than the reproductive organs, is the lateral outgrowths from the body walls immediately back of the head. The original description by Verrill ('92) records a different state of development of these appendages in each of the four specimens which he had for study. The smallest specimen had short, blunt tentacles, only about half as long as the breadth of the head; another had slender tapering tentacles as long as the diameter of the body, while in the largest individual they were more than twice as long as the width of the head, with slender, lash-like, somewhat coiled tips.

A specimen which has more recently come into our hands has the tentacles about midway between the extremes described by Verrill, or one and one-half times as long as the width of the head (fig. 1). We have found, as was to have been expected, that all these five specimens are males, and that those with the tentacles most highly developed have the spermaries in the most advanced stages, while those with the short tentacles are still immature.

It seems obvious, therefore, as has already been pointed out by Brinkmann ('12), that these appendages represent secondary

sexual characters and reach their full development only with the sexual maturity of the animal.

In the females, as stated above, the tentacles are entirely lacking, such individuals having been described by Verrill ('92) under a separate genus and species, *Hyalonemertes atlantica*.

As shown in figure 1, the tentacles arise as lateral outgrowths of the body walls immediately back of the head. The sections show, as described by Cravens and Heath ('06) for *N. pelagica*, and as Bürger ('09) also explains, that the tentacles consist mainly of strong muscle bundles directly continuous with the muscular layers of the body wall. The musculature is covered by a well-developed basement layer (fig. 13, *bm*) similar to, but thicker than; that of the body walls. The epithelial covering has been entirely dislodged, as in the case of the body integument.

The musculature of the tentacles consists of four distinct sets of muscles instead of the three in the body walls. These are: *a*) the outer longitudinal muscular layer (fig. 12, 13, *lm'*), which is a direct continuation of the circular musculature of the body but which runs throughout the tentacle longitudinally; *b*) the circular muscular layer (*cm'*), of great thickness, which is derived from the longitudinal body muscles, which change their direction, first becoming diagonal and then assuming a circular or spiral course through the whole extent of the tentacle; *c*) the inner longitudinal muscular layer (figs. 12, 13, *ilm*) which arises mainly from the circular muscles of the body; the latter become much thicker at the base of the tentacle, sending off not only the outer longitudinal muscles of the tentacle, but also oblique bundles which pass through the longitudinal muscles of the body wall to form this internal longitudinal layer of the tentacle; *d*) the dorsoventral bundles (*dvm*) which are here very highly developed and pass at right angles through the inner longitudinal muscles to connect the dorsal and ventral portions of the circular tentacular muscles. As shown in figure 13, these dorsoventral bundles are continuous laterally with the circular muscles, the individual bundles separating from this more and more toward the median line of the tentacle.

The parenchyma is limited to relatively small areas which lie between the multitudinous muscle bundles. Interspersed among the areas of parenchyma are very numerous blood lacunae.

Conspicuous nerves leave the lateral nerve cord in the region of the tentacle and extend the entire length of the organ, sending numerous branches to the musculature and presumably also to the integument. There are two main tentacular nerves, one lying dorsally and the other ventrally in the median vertical plane.

Function. The presence of these large nerves led Bürger ('09) to believe that the function of the tentacles was sensory, and this office they probably have to a certain extent. Their main function, however, seems obvious, now that we know that they develop only in the male sex, and there only coincidentally with the sexual maturity of the individual.

The tentacles are, we must remember, associated with cephalic gonads small in number, the spermatozoa of which are used to fertilize a very small number of eggs in the female. Hence with this restriction in the number of possible offspring from a pair, there must be the greatest requirement that fertilization should always be successful. Nature cannot in this case afford such a waste of sexual products as occurs in other nemerteans where thousands of times as many gametes are produced by each individual. Therefore comes the advantage of an act of sexual union to provide the greatest assurance of insemination.

The tentacles meet this requirement in providing the sexually mature male with a pair of strong muscular appendages which enable him to cling to the female until her eggs have been fertilized. This clinging instinct doubtless accounts for the fact that the males are so much oftener secured than are the females, for the former use their tentacles to cling to other objects, such as nets and fishing lines. In this instance the tentacles are of course unadaptive, but that does not invalidate their general adaptiveness, for nets and lines are not natural to the animal's environment.

PROBOSCIS AND PROBOSCIS SHEATH

Proboscis sheath. The opening of the rhynchodeum is on the dorsomedian part of the tip of the head (fig. 17). The rhynchodeum (*ro*) is very short, reaching less than half way to the brain, and is lined with highly columnar epithelium which, in some of the specimens, has become entirely dislodged. It is surrounded by interlacing circular and muscular fibers, which in the contracted state make a rather thick layer, but which doubtless becomes very thin when the chamber is open for the passage of the proboscis. Radiating in all directions from the rhynchodeum are delicate muscle bundles, homologous with the dorsoventral muscles of the body, which hold this organ in its proper position in the parenchyma which surrounds it. At the point where the proboscis is attached these muscles become enormously increased. The proboscis sheath extends through about nine-tenths the length of the body, terminating just beyond the base of the caudal fin (fig. 1).

The rhynchocoel varies greatly in diameter according to the state of contraction of the body. The difference in this respect between the voluminous cavity in the contracted specimen shown in section in figure 10 and the narrow tube in the well-extended specimen illustrated by figure 1 is very striking.

The wall of the proboscis sheath remains thin and with but few circular muscles for some distance back of the brain, but gradually increases in thickness posteriorly (figs. 10, 17). Excepting in its anterior portion, where the proboscis sheath shows more or less distinct circular and longitudinal muscular layers, the musculature of this organ consists of interlaced circular and longitudinal muscular fibers similar to those found in well-known species of *Depanophoridae*.

Instead of terminating in the body musculature, as Verrill ('92) erroneously states, the proboscis sheath gradually becomes extremely slender toward its posterior end and terminates in a great mass of body parenchyma, entirely free from even dorsoventral muscles.

Proboscis. The length of the proboscis nearly equals the length of the body when well extended (fig. 17), but in contracted speci-

mens shows numerous convolutions. In the vast majority of specimens of this and related species the proboscis has been discharged from the body and lost before preservation. In two of specimens which we have studied, however, this organ was intact, and in sections shows the characteristic Metanemertean structure (figs. 10 and 11).

Between the outer, thin, flattened epithelial layer (fig. 11, *ep*) and the inner layer of columnar epithelium (*ep'*) there are the usual outer and inner (*cmp*, *cmp'*) layers of circular muscles and a much thicker intervening layer of longitudinal muscles (*lmp*), the latter being divided by the nervous layer (*pn*) into two layers of approximately equal thickness. In the nervous layer are about twenty rather well-demarcated proboscidial nerves connected by a nervous plexus, giving the proboscis a highly developed innervation. In 21 specimens studied by Brinkmann ('17, '17a) the number varied from 18 to 24.

The two specimens in which the proboscis was present were very carefully studied after clearing in suitable medium, and one was sectioned. The armature was found to consist of a minute, slender, curved or hook-shaped basis, bearing a row of about ten to twelve tiny, conical stylets.

At its posterior end the proboscis is provided with a retractor of such strength that when the proboscis is spasmodically extended, instead of being normally everted, the retractor and the terminal end of the proboscis remain attached to the sheath. Cravens and Heath ('06) found the same condition in *N. pelagica*. The attachment of the retractor to the proboscis sheath lies some little distance in front of the posterior end of the latter.

ALIMENTARY CANAL

Mouth and proboscis openings are widely separated, as shown in figure 17, the former opening on the ventral and the latter on the dorsal side of the anterior end of the body. The buccal cavity has the surface epithelium thrown into deep folds (fig. 17, *m*) which enable the lips to be enormously distended during the ingestion of food. The narrow oesophagus passes backward

less than half way to the brain commissures (figs. 14, 17), where it merges into the stomach (*st*), likewise a narrow tube; from the stomach the slender pylorus (*pyl*) leads backward for a short distance and opens, as in other related forms, into the dorsal wall of the intestine (*in*). The intestine extends anteriorly beneath the pylorus and stomach as a broad pouch, or caecum (*ic*), which reaches forward to the posterior brain region and sends paired lateral diverticula dorsally above the gonads and the proboscis sheath. These diverticula (*icd*, figs. 14, 17) are broad, irregularly lobed, and are so closely placed that they leave but little of the body parenchyma between them. The diverticula of the caecum are scarcely to be distinguished from those of the intestine proper, but tend to be somewhat more irregular in outline by having rather deeper lobes.

The intestine, with about sixty pairs of broad diverticula, extends posteriorly nearly to the end of the body. Figures 1 and 10 show how completely these intestinal diverticula fill the space within the body walls. Indeed, in the more contracted specimens the diverticula are much more closely crowded together than is shown in figure 1. In the living animal, on the other hand, they are doubtless more widely separated than here shown, for, as stated above the best-preserved specimen has contracted in alcohol to one-fourth its original volume, and this shrinkage must have come largely from the parenchyma which separates the intestinal diverticula. The diverticula are all irregularly lobed, but not branched, both vertically and horizontally, and for the most part appear more or less distinctly bilobed distally when seen from the dorsal surface (fig. 1). They conform to the general outline of the body except in the regions of the tentacles and the horizontal lateral fins, as well as in the caudal fin, where lateral extensions of the body walls beyond the general outline of the body occur (figs. 1, 8, 9). Immediately anterior to the caudal fin the diverticula become shorter and gradually decrease in length posteriorly (fig. 1) until they disappear in the short and narrow rectum (figs. 1, 9) which opens at the very extremity of the body.

VASCULAR SYSTEM

The blood vascular system has been so fully described by Cravens and Heath ('06) for *N. pelagica* and correctly, although in less detail, by Bürger ('09) for *N. minima*, that it is unnecessary for us to state further than that we have verified their descriptions. Figure 1 shows the anterior anastomosis (*cv*) in front of the brain, the junction of lateral and median vessels just back of the brain, and the posterior anastomosis of median (*mv*) and lateral (*lv*) vessels above the posterior portion of the intestine in the caudal fin.

Nephridia seem to be lacking in all bathypelagic species.

NERVOUS SYSTEM

The brain and lateral nerve cords are essentially as described for *N. pelagica* by Cravens and Heath ('06). Both dorsal and ventral commissures are well developed (fig. 14). Near their origin from the brain lobes the lateral nerves bend sharply outward toward the lateral margins of the body, whence they continue backward in a ventrolateral position beneath the intestinal diverticula and directly lateral to the blood-vessels (figs. 1, 10) nearly to the extremity of the body. In sexually mature males the lateral nerves (fig. 6, *ln*) are forced dorsally from their normal positions by the fully developed spermaries, resuming their ventrolateral positions immediately posterior to the gonads. In the posterior half of the caudal fin they bend dorsally above the most posterior intestinal diverticula to unite in a well-developed commissure (figs. 1, 8, *ln*) above the intestine immediately anterior to the rectum.

The nerves leading to the tissues of the head, to the proboscis, to the lateral tentacles, and to the body musculature are large and are quite conspicuous in sections as they pass through the body parenchyma.

The fibrous central core of the lateral nerves is divided distinctly into a smaller dorsal and a larger ventral portion, well separated by ganglion cells (figs. 6). The dorsal core gradually becomes proportionately smaller than the ventral the farther

back it extends, being about one-third the diameter of the ventral core in the middle of the body and only one-tenth of this diameter near the posterior end of the body.

These worms, in common with many pelagic nemerteans, lack both cerebral and frontal sense organs.

REPRODUCTIVE ORGANS

The sexual dimorphism of the species of the genus *Nectonemertes* is described above (page 459), and is more fully discussed in a recent paper by Coe ('20).

Spermaries

The spermaries, as in most other truly pelagic nemerteans, are limited to the head, there being fifteen or more in an irregular elongated group situated ventrolaterally on each side of the head (fig. 1). These were correctly described by Verrill ('92), but their true nature was not recognized by him. He suggested with some hesitation that they might possibly be special sense organs. In each of these groups the spermaries appear to be roughly arranged in two longitudinal rows, of which one lies distally and the other medially to the lateral nerve cord (fig. 1), but there is great irregularity in this respect, due to the crowding of the spermaries and the irregularities of the contraction of the body. Sometimes a partial third row of three or four spermaries lies internal to the posterior end of the other two, as shown on the right-hand side of figure 1. The opening of each gonad is situated on the summit of a small papilla, which in some cases rises well above the surface of the body wall. An extreme development of these papillae has been described by Brinkmann ('12) for *Bathynectes murrayi*, where the efferent ducts from the spermaries extend from the body as long, slender penes.

In transverse section of the head of a fully matured individual (fig. 6) the spermaries fill the major portion of the space within the body walls, crowding the diverticula of the intestinal caeca (*icd*) close up against the dorsal wall of the body and almost obliterating its lumen. The lateral nerve cord (*ln*) and the blood-

vessels (*lv*) are likewise transposed from their normal lateroventral positions into the dorsal half of the body.

Each gonad is surrounded by a massive wall of circular muscular fibers (figs. 2, 3, 6), as described by Cravens and Heath for *N. pelagica*. The individual muscle fibers appear to run spirally about the gonad. They are irregularly arranged in groups of from three to ten with nearly all their spindle-shaped nuclei (fig. 3, *n*) next the inner surface of the wall. In transverse section the nuclei are oval in outline and occupy the enlarged inner borders of the muscle fibers.

The general shape of the gonad resembles a retort with its neck directed ventrally and laterally. The germinal cells occupy only the body of the retort-shaped organ, the neck serving as a duct for the discharge of the spermatozoa. At about the middle of the neck of the retort (figs. 2, 3, 6) the muscular wall of the gonad is terminated sharply by a narrow constriction which marks the end of the gonad proper. The opening in this constriction (fig. 3, *o*) leads into a spacious chamber, the wall of which consists of a single layer of cuboidal epithelium. This chamber serves as a seminal vesicle (fig. 3, *sv*), and into it the ripe spermatozoa collect previous to their discharge from the body.

The opening of the seminal vesicle to the exterior remains closed except at the moment of discharge, and the ripe spermatozoa collect in compact bundles (figs. 3, 7, *spz'*) in the efferent duct. The hundreds of spermatozoa of a bundle all lie parallel, with their heads directed toward the opening. The genital pore is guarded by a pair of lips (fig. 2) on the summit of the small papilla already mentioned.

In the body of the gonad there is a small central lumen filled to a great extent with ripe spermatozoa (fig. 3, *spz*), with great masses of germinal cells in the various phases of spermatogenesis peripherally. As in most similar cases, the nuclei of these cells are conspicuous, but the cell boundaries are indistinct.

Just internal to the massive circular musculature occur the scattered oval nuclei of the spermatogonia (*spg*) while the nuclei of the spermatocytes are arranged in groups. Those of the primary spermatocytes are large and spherical (*spc*), while those of the

secondary spermatocytes (*spc'*) are naturally much smaller. The spermatozoa in their earlier stages are clustered together with their heads imbedded in large nurse cells. In the more mature spermatozoa (*spz*), which occupy the lumen of the gonad, the slender heads of a hundred or more may lie side by side with their tails forming a compact wavy bundle.

The developmental stages of the peculiar structures described above for the fully mature gonad are well shown in some of the immature spermaries and are illustrated in figures 15 and 16. In figure 15 the seminal vesicle (*sv*) is seen to consist of a pointed outgrowth from the gonad which penetrates the body wall only as far as the basement layer. The single layer of cuboidal epithelium which makes up the wall of the seminal vesicle here extends into the body of the gonad for a short distance and then terminates abruptly in the muscular layer (*m*). Spermatogonia (*spg*) lie next the muscular wall, while the body of the gonad is filled with spermatocytes (*spc*). Figure 16 is a tangential section of the gonad, showing the muscles and their slender nuclei cut lengthwise and the seminal vesicle cut transversely.

The peculiar modifications of the spermaries and their ducts indicate a provision for the conservation of the genital products and an assurance of insemination. The significance of the seminal vesicles, found in no other genus of nemerteans, so far as known, is obviously to supplement the spermaries in storing the ripe spermatozoa and probably also to aid in their expulsion. The powerful musculature indicates a forcible ejection of the spermatozoa during the act of pairing. And, finally, it is perhaps reasonable to conceive of the genital papillae as being capable of elevation, with the aid of the seminal vesicles, far above the surface of the body walls, so that they may be placed directly against or even into the oviducts of the female. The contraction of the musculature of the gonad might then, conceivably, force the spermatozoa into direct contact with the egg or pair of eggs in the ovary. The two worms are meanwhile held in close contact by the muscular tentacles of the male.

Brinkmann's discovery ('12) that in *Bathynectes* the sperm ducts open on the tips of long, slender processes, which he calls penes, supports this hypothesis.

Ovaries

The ovaries in *Hyalonemertes atlantica*, which we now recognize as the female of *Nectonemertes*, are arranged in a single row along each ventrolateral border of the body in the immediate vicinity of the lateral nerves. Each ovary produces but one or two eggs of large size, as in other bathypelagic nemerteans.

It is very evident that Verrill ('92) saw and described these ovaries correctly as he did nearly all the organs that could be studied without the aid of sections. Unfortunately, however, this keenest of observers apparently made an error in transcribing his notes and placed the description of the ovaries of *Hyalonemertes* in the diagnosis of *Nectonemertes* (p. 448). As his specimens of *Nectonemertes* are still available for study and show no such organs as he mentions, there is reasonable certainty that the explanation given is correct.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Nectonemertes mirabilis

1 Mature male cleared in cedar oil after staining with borax carmine, showing the internal anatomy, with the flattened caudal fin at the posterior end of the body, the horizontal fins, and the tentacles. The slender, slightly convoluted proboscis is attached posteriorly near the end of the proboscis sheath. Anteriorly the specimen shows the cephalic loop (*cv*) of the lateral vessels above the rhynchodeum, the relation of dorsal ganglia (*dg*), lateral nerves (*ln*), the cephalic nerves, and the number and position of the retort-shaped spermaries (*sp*). Near the posterior end of the body is seen the common union of the two lateral blood-vessels (*lv*) and the median vessel (*mv*), and the posterior anastomosis of the lateral nerve cords (*ln'*) above the intestinal diverticula (*id*). $\times 6$.

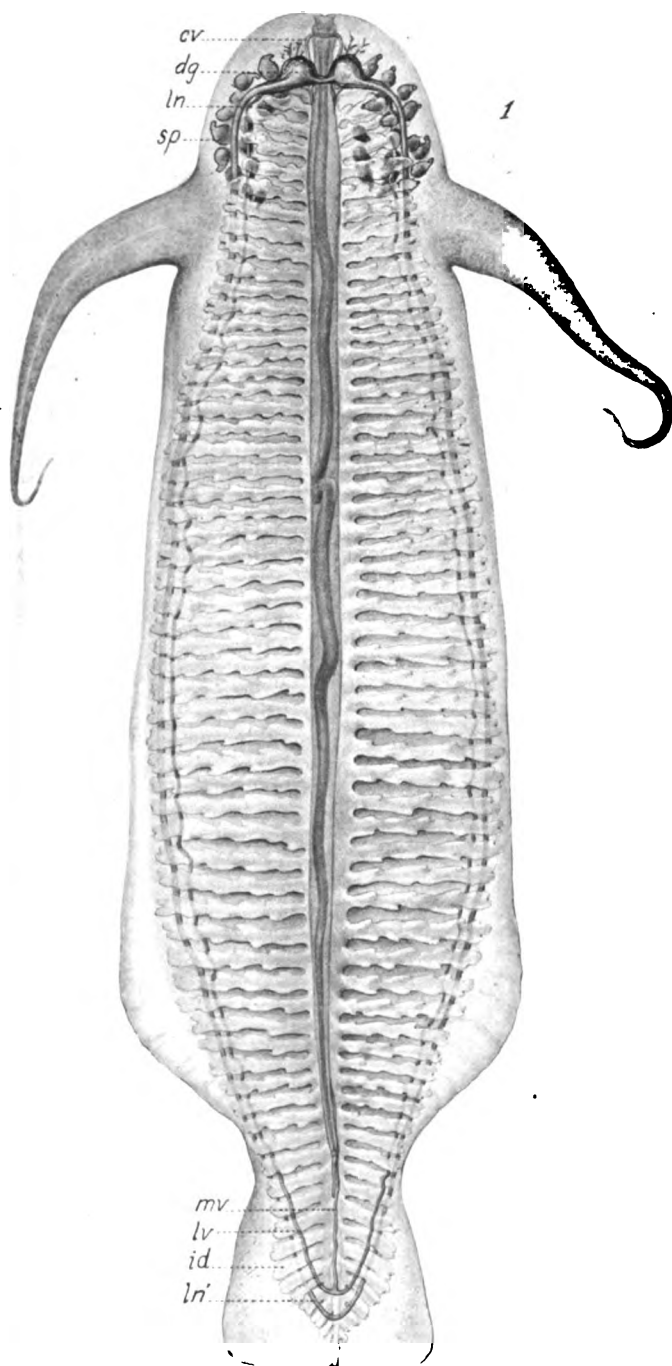


PLATE 2

EXPLANATION OF FIGURES

Nectonemertes mirabilis

2 Surface view of a mature spermary, showing the thick layer of spiral muscular fibers surrounding the spermary proper and the epithelial wall of the seminal vesicle, the latter leading to the minute external opening on the ventrolateral border of the head. $\times 60$.

3 Optical section of a spermary, with its seminal vesicle and spermatic duct. The thick muscular columns (*mc*) of the spiral musculature surround the germinal cells, which occur in all stages of development; *spg*, spermatogonia; *spc*, primary spermatocytes; *spc'*, secondary spermatocytes; *spl*, spermatids; *spz*, developing spermatozoa collected in bundles about the nurse cells; *o*, opening from spermary into the seminal vesicle (*sv*) with its thin epithelial wall; *spz'*, mass of mature spermatozoa ready to be discharged through the spermatic duct which opens to the exterior by a minute pore in the basement membrane (*bm*) of the body wall; *n*, nuclei of muscle fibers. $\times 220$.

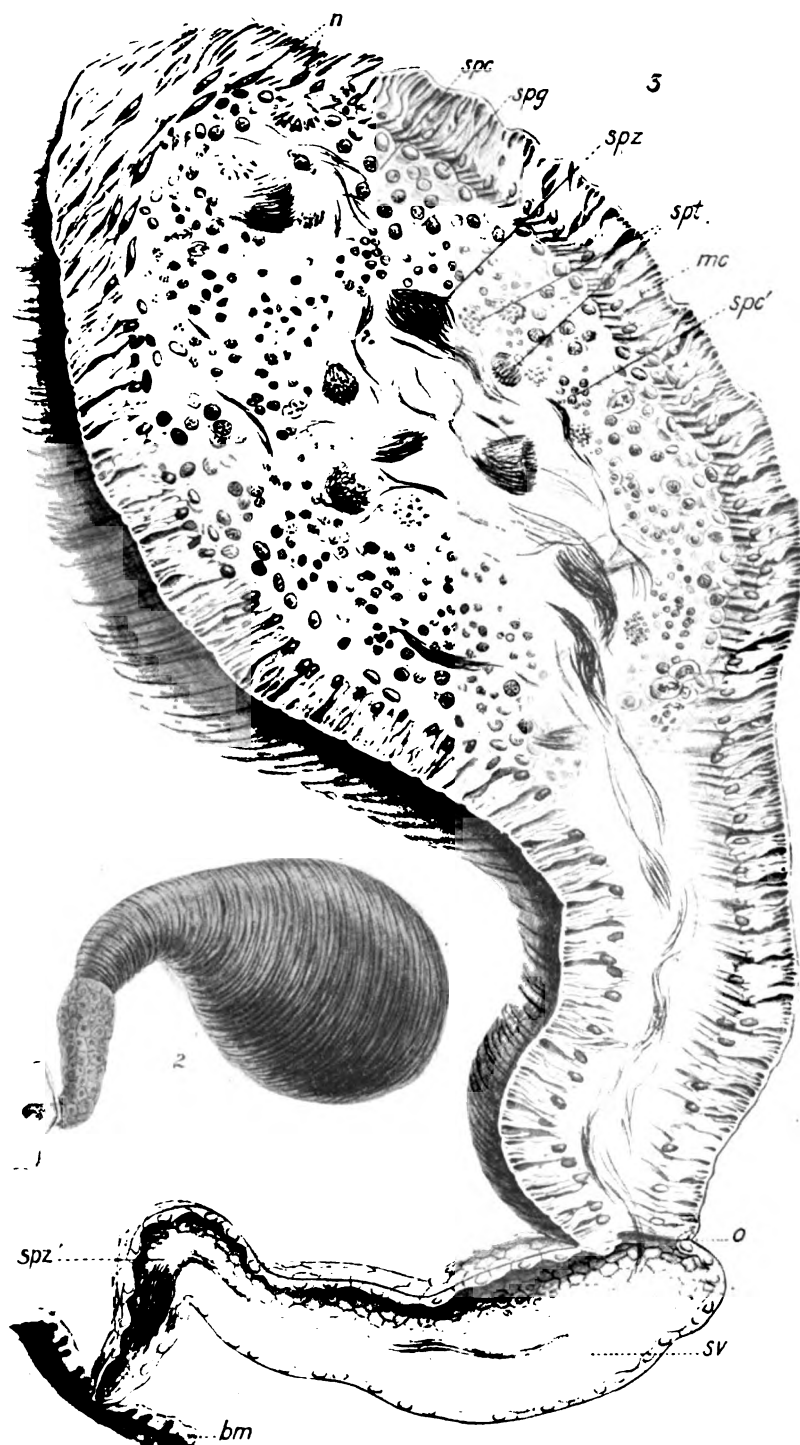


PLATE 3

EXPLANATION OF FIGURES

Nectonemertes mirabilis

4 Ventral view of male individual, showing the thin lateral margins, horizontal fins, and caudal fin. The position of the mouth is also shown. $\times 1\frac{1}{2}$.

5 Lateral view of body, showing its adaptation for swimming. $\times 1\frac{1}{2}$.

6 Portion of transverse section of body anterior to tentacles, showing sections of four spermaries (*sp*), cut in different planes. In two of the spermaries the seminal vesicles (*sv*) and spermatic ducts are shown. The spermaries encroach to such an extent on the other tissues as to force the lateral nerve (*ln*) and the diverticula of the intestinal caecum (*icd*) close against the dorsal wall of the body; *lv*, lateral blood-vessel; *par*, parenchyma; *mc*, muscular layer of spermary; *lm*, longitudinal, and *cm*, circular muscular layers of body wall; *bm*, basement layer. The epithelial covering of the body is missing. $\times 70$.

7 Terminal portion of seminal vesicle and spermatic duct showing mass of mature spermatozoa (*spz'*) ready for discharge through genital pore. $\times 200$.

8 Half of transverse section through caudal fin, showing posterior commissure of lateral nerves (*ln'*) between and above intestinal diverticula (*id*) and the series of dorsoventral muscle bundles between dorsal and ventral body walls. $\times 50$.

9 Portion of transverse section through posterior extremity of body, showing extreme development of dorsoventral muscles in the caudal fin, with their nuclei lying midway between the two ends of the fibers; *r*, rectum. $\times 50$.

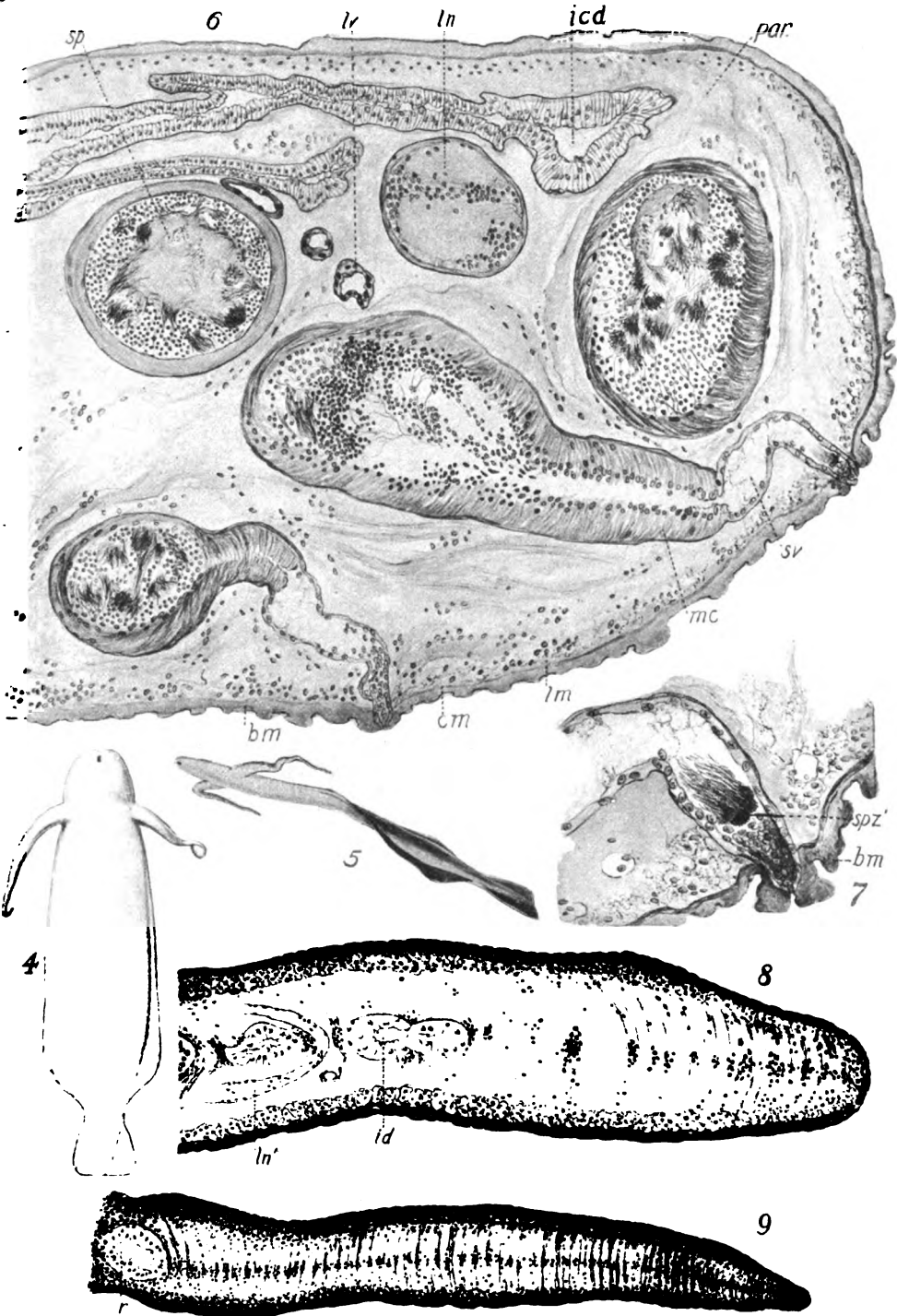


PLATE 4

EXPLANATION OF FIGURES

Nectonemertes mirabilis

10 Transverse section through middle of body, showing proboscis (*p*) in its sheath (*ps*) and the extensive development of the intestinal diverticula (*id*); *cm*, circular muscular layer; *lm*, longitudinal muscular layer; *ln*, lateral nerve; *lv*, lateral blood-vessel; *rc*, rhynchocoel. $\times 116$.

11 Transverse section of proboscis, showing the twenty proboscidial nerves (*pn*); *cmp*, outer circular muscles; *cmp'*, inner circular muscles; *ep*, outer epithelium; *ep'*, inner epithelium; *lmp*, longitudinal muscles. $\times 96$.

12 Portion of longitudinal section of tentacle, showing its musculature; *cm'*, circular muscular layer; *dvm*, dorsoventral muscles; *ilm'*, inner longitudinal, and *lm'*, outer longitudinal muscular layers; *bm*, basement layer. $\times 180$.

13 Transverse section of tentacle; lettering as in figure 12. $\times 180$.

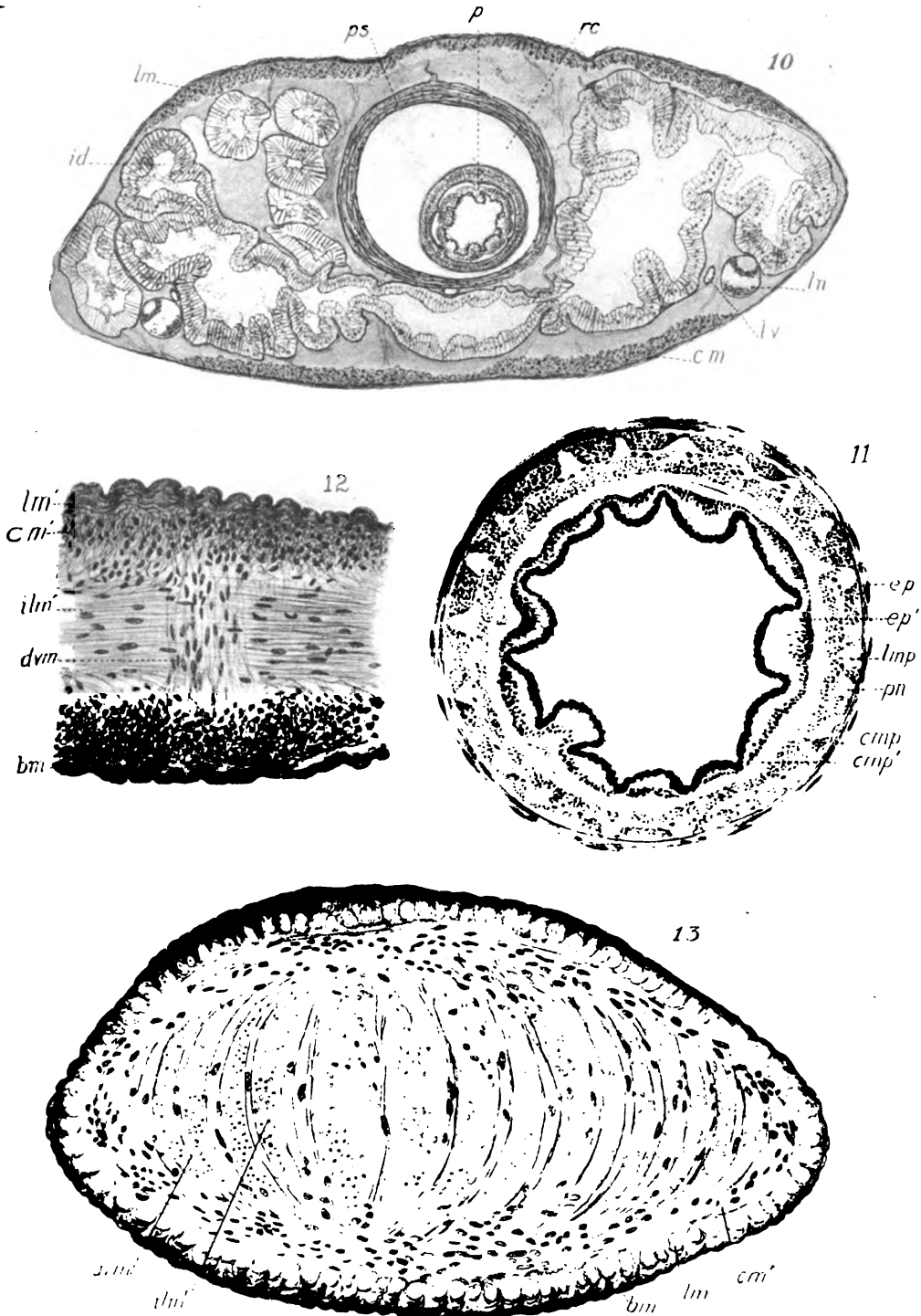


PLATE 5

EXPLANATION OF FIGURES

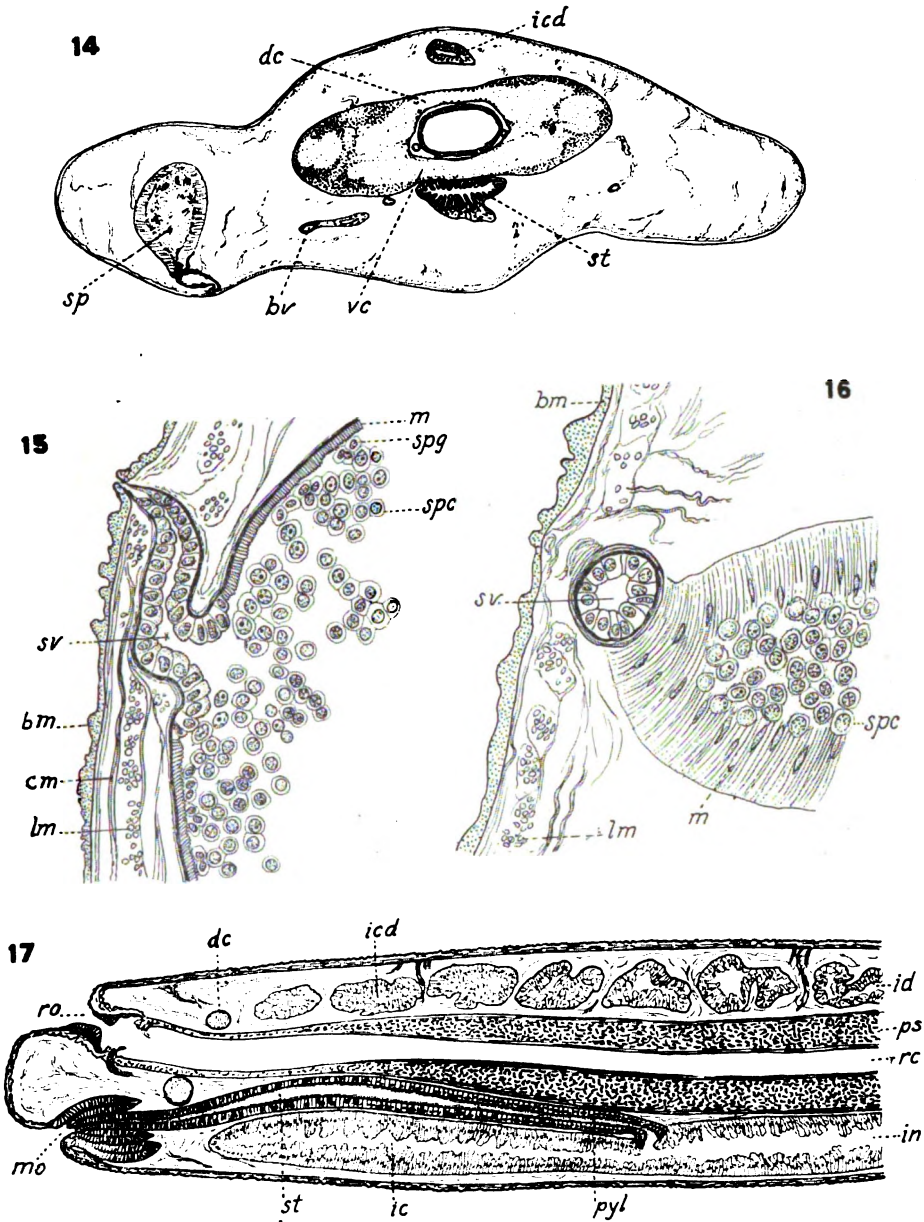
Nectonemertes mirabilis

14 Transverse section through head of mature male; slightly reconstructed from adjacent sections, showing brain lobes with dorsal (*dc*) and ventral (*vr*) commissures; stomach (*st*), immediately beneath ventral commissure, and a single lobe (*icd*) of the anterior diverticulum of intestinal caecum. A fully mature spermary (*sp*) is shown, with seminal vesicle leading to ventral surface of head. $\times 24$.

15 Portion of a transverse section of body, showing an immature spermary with developing seminal vesicle (*sv*) with its simple epithelial lining extending through body wall. The muscular wall (*m*) of the gonad encloses the developing spermatogonia (*spg*) and spermatocytes (*spc*); *cm* and *lm*, circular and longitudinal body musculatures, respectively; *bm*, basement layer.

16 Portion of transverse section of body, showing tangential section of a gonad with the musculature (*m*) cut longitudinally, and the seminal vesicle cut transversely. Lettering as in figure 15. $\times 300$.

17 Sagittal median section of anterior portion of body. Slightly reconstructed from adjacent sections, showing opening of the rhynchodeum (*ro*), attachment of proboscis sheath (*ps*), mouth (*mo*) with its folds of epithelium, leading through a short oesophagus to stomach (*st*), and the latter to pylorus (*pyl*); *ic*, intestinal caecum; *icd*, diverticula of same; *in*, intestine; *id*, intestinal diverticula; *dc*, dorsal brain commissure, the ventral commissure being also shown above stomach $\times 24$.



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El tegumento de *Necturus maculosus*.

En el presente trabajo el autor dá a conocer una revisión mas o menos completa del tegumento de *Necturus*. Una verdadera capa córnea existe solamente en las manos y piés. En otras regiones las superficies libres de las células epidérmicas poseen espesas placas apicales o márgenes cuticulares. Estos últimos presentan una estriación perpendicular y son, en parte, productos de secrección. Alrededor de los labios y en el pliegue gular existen células caliciformes. Las células de Leydig son abundantes. También existen cromatóforos piramidales y ramificados. A una luz intensa los melanóforos de la dermis se dilatan completamente. En la oscuridad generalmente se contraen. La temperatura y el color del fondo pueden modificar los cambios de color.

Las glándulas dérmicas son de dos clases: granulares y mucosas, distinguiéndose por el caracter y reacciones colorantes de su secrección. Las primeras están rodeadas de una pared muscular. En las glándulas mucosas por el contrario, no se encuentran jamás músculos. Ambos tipos de glándulas se desarrollan como invaginaciones de la capa germinativa de la epidermis y no pueden distinguirse entre sí en los primeros estados. Las células granulares no pasan nunca por un estado mucoso. En algunos casos se encuentran también glándulas mixtas.

Durante la elaboración y expulsión de la secrección granular se destruye el epitelio. La regeneración tiene lugar mediante una invaginación de nuevo epitelio derivado de la region intercalar y de la epidermis, colocada superiormente. El modo de renovarse las glándulas mucosas no es manifiesto. La musculatura y el epitelio de las glándulas granulares poseen una inervación directa. Sobre la superficie externa del epitelio secretor de las glándulas mucosas existen fibras ramificadas.

Translation by José F. Nonidez
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THE INTEGUMENT OF NECTURUS MACULOSUS¹

A. B. DAWSON

SIX PLATES (THIRTY-SEVEN FIGURES)

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INTRODUCTION

A. Plan and purpose

The skin and especially the large skin glands of Amphibia have been the subjects of much study and discussion since Ascherson's investigations on the frog in 1840. A survey of the literature on these subjects since that date reveals a fundamental ground-plan for the integument of all Amphibia. In many groups, however, peculiarities of structure and function of great interest appear, doubtless called forth by changes in habitat and manner of living. The failure of many investigators to realize that actual differences might exist has led to much unnecessary controversy. Still it is obvious that, even in cases where identical structures have been described, differences of interpretation are bound to occur. In this paper there will be no attempt to extend the writer's interpretations of conditions found in *Necturus* to the whole class of Amphibia. Rather, an effort will be made to point out wherein *Necturus* resembles the other members of this class and wherein it is specifically different.

This particular study was undertaken originally to determine the relation, if any, between the two apparently distinct types of dermal glands in the *Necturus* skin. During the early stages of the work other interesting problems relating to epidermal and dermal elements presented themselves, and it was decided to extend the work to include the entire integument of the animal.

The other interesting problems of the epidermis are connected chiefly with the so-called cuticular margin, the unicellular glands, and pigmentation. Also the structure of the dermis, its relation to the epidermis, the distribution of elastic tissue, dermal pigmentation, and changes in coloration are described. As already intimated, the major part of the work is devoted to the large glands, of epidermal origin, imbedded in the dermis. In these the principal questions concern, 1) the identity of the granular and mucous types; 2) the nature of the secreting epithelium; 3) the elaboration and expulsion of the secretion, and, 4) the subsequent fate of the glands. Some attention is also paid to the innervation of the various elements.

It is a pleasure to acknowledge here my deep indebtedness to Dr. H. W. Rand, at whose suggestion this research was undertaken and under whose helpful supervision it was carried out. I wish also to thank Dr. E. L. Mark, director of the Zoological Laboratory of the Museum of Comparative Zoology at Harvard College, for many privileges and courtesies extended to me.

B. Material and methods

The material studied was obtained from healthy animals sent to Cambridge from Venice, Ohio. All were full-grown with the exception of one larva 10 cm. long.

Animals were either anaesthetized or killed in a 0.2 per cent solution of chloretone, which gave better results than either chloroform or ether, since these reagents are very irritating and usually caused expulsion of considerable secretion. For the study of the glands most of the tissue was taken from the thin dorsal and ventral regions of the edges of the tail. This was advantageous for several reasons: first, because the glands are unusually well developed here; secondly, because little mechanical disturbance was necessary in removing a portion of integument, and, thirdly, because the same animal could be used many times, as the surface exposed by cutting was very small. In fact, pieces were clipped from the tail of unanaesthetized animals without causing any apparent discomfort. Tissues obtained in this manner and fixed immediately permitted a study of the glands in a practically normal condition.

Many common fixing fluids were used. Kleinenberg's fluid gave the most satisfactory results, and a large proportion of the material sectioned was fixed in this way. Tissue so prepared did not become hard, took all stains readily, and the histological preservation was good. Bouin's, Flemming's (weak), Gilson's, and Zenker's fluids were employed occasionally. The granular secretion was best preserved by using a 2.5 per cent solution of formaldehyde or a saturated solution of corrosive sublimate.

For microscopic study serial paraffin sections (10 μ thick) were cut in two planes, one at right angles to, the other parallel to, the body surface. Unstained preparations made from free-hand

sections of fixed and fresh material also proved helpful. The fresh material was examined in glycerin or normal salt solution. For the determination of the general distribution of the various elements, relatively large strips of skin were dissected off, fixed, cleared, and mounted.

Little difficulty was encountered in the preparation of serial sections. Some trouble arose due to overhardening, but it did not become necessary to resort to the use of celloidin. The hardening was avoided by using small amounts of tissue and reducing to a minimum the length of treatment in the stronger alcohols, xylol, and paraffin. Pieces of integument, 5 to 8 mm. square, cleared of all underlying muscle, were placed in Kleinenberg's fluid from two to two and one-half hours and then washed in 70 per cent alcohol for twenty-four hours. After the excess fixing fluid had been removed, the tissue was dehydrated, being left in 90 per cent alcohol for twenty minutes and in absolute alcohol for twenty-five minutes. This seldom failed to give complete dehydration, and clearing in xylol was accomplished in about fifteen minutes. Good infiltration was then obtained by using successively soft and hard paraffin, leaving the tissue twenty minutes in each.

A large number of stains were used, separately and in combination. Ehrlich's or Delafield's haematoxylin, contrasted with eosin or some common plasma stain, was used for ordinary routine work. Other stains, such as Heidenhain's iron haematoxylin, Weigert's resorcin-fuchsin for elastic tissue, haematoxylin counterstained with Van Gieson's mixture, and Mallory's connective-tissue stain, were found useful in the differentiation of special parts of the integument. Thionin was generally employed as a specific stain for the mucous secretion, but a slight over-staining in either Delafield's or Ehrlich's haematoxylin followed by 'bluing' in an ammoniacal solution, was usually sufficient to color the contents of the slime glands. In the study of the nerves of the glands and epidermis two methods were relied upon, namely, the silver nitrate-hydroquinone method of Cajal and Ranson's pyridin-silver nitrate-pyrogalllic acid method. The last named gave the better results.

C. General observations

The large perennibranchiate urodele, *Necturus maculosus* Raf., on which these studies were made, has the typical soft skin of the water-inhabiting Amphibia. The surface of the body is smooth, exhibiting no modifications or prominences such as appear in other Amphibia due to the massing of the large glands in the dermis. In a shallow aquarium, in clear water, the numerous pinhole-like openings of the individual gland ducts are visible even to the naked eye. No secretion can be detected on the surface of the body while the animal is resting quietly. When picked up a clear slimy secretion immediately appears and the animal quickly wriggles from the grasp. It is only when violently stimulated mechanically, chemically, or electrically that the gray granular secretion is expelled. That which is first discharged floats away free in the water, but the later discharge is held imbedded in an ever-increasing amount of clear slime which envelops the entire surface of the animal.

In favorable light the lateral-line system can be clearly distinguished on the living animal, the several tracts, above and below the eyes, over the snout and jaws, and along the sides of the body, appearing as series of short broken lines. This system has already been completely mapped for *Necturus* by Kingsbury ('95), and studied histologically by Takahashi ('09).

The color of *Necturus* is so variable that no complete description will be attempted. The pattern is produced by a mingling of black and yellow pigments on a white background. Above, the animal usually appears a dark uneven brown with a more or less pronounced black mottling; below, the pigmentation is less dense and the pattern more regular. The mottling is caused by a grouping of the large black chromatophores of the dermis. Around these black areas a narrow yellow margin can often be distinguished. Occasionally the spots run together to form black bands of considerable length, the most common and conspicuous being a pair extending along either side of the head from the nostrils to a point immediately anterior to the large plume-like gills. In some cases, however, the mottling is entirely lost and the upper surface presents a finely granular appearance due to the equal distribution of the melanophores and xanthophores.

The ventral surface of the body exhibits considerable variation in pigmentation. Often it is completely pigmented, but not so densely as the dorsal surface. Sometimes there is a distinctly lighter median area; at other times, a sharply defined narrow white line is present. In a few animals the entire venter, from the cloaca to the gular fold, is white, lightly flecked by a few scattered melanophores, which are usually distributed along the lateral margins. The color pattern of the larvae is strikingly different from that of the adults. The most conspicuous feature in larval pigmentation is the two broad dorsolateral bands of pure yellow which run the entire length of the body and extend even to the tip of the tail. The contrast of yellow and black, which is vivid in the young animals, becomes subdued in the adults, and in some cases disappears almost completely.

EPIDERMIS

A. General description

The epidermis is a stratified epithelium having from two to eight layers of living cells (figs. 1, 6, 12). The cells of the lower layer, in contact with the dermis, are tall columnar and constitute the germinative layer. The several layers above this are composed of the ordinary polygonal cells, showing no differentiation in size or form. Resting upon these transitional layers is a specialized stratum of cells known as the cuticular layer, the cells of which have on their outer surfaces a peculiarly differentiated zone generally referred to as the cuticular margin (fig. 1).

The thickness of the epidermis, except in the gular fold region (fig. 16), varies but slightly throughout the entire body. The greatest thickness is found on the hands and feet, where there is developed a distinct stratum corneum (fig. 11) not found elsewhere. On the head and body practically no differences in thickness appear. On the dorsal and ventral edges of the tail there is a small reduction in number of the cell layers, but the maximum reduction occurs in the gular fold region, where the epidermis is only two or three cells thick. Concentric with the

openings of the ducts from the granular glands, there usually is a slightly thickened zone within which the layer thins as its surface slopes down to the mouth of the duct (fig. 6). In such places the lower surface of the epidermis projects into the dermis, as if drawn down by a tension exerted by the large underlying gland. The smaller mucous glands produce no such disturbances in the epithelium (fig. 12).

In general, the form of the epidermal nucleus is determined by the shape of the cell in which it lies. The chromatin is arranged in an irregular network and no nucleoli are present. The cytoplasm is usually homogeneous in appearance, but in many cells the nucleus is surrounded by a clear zone (Nordenskiöld, '05, *Bufo*). In a few preparations there appeared a concentration of densely stained cytoplasm at the deeper poles of the nuclei. Prowazek ('01) describes a similar condition in salamander larvae and considers it an evidence of degeneration.

The occurrence of mitosis in any of the cells of the amphibian integument except the germinative layer has been denied by many investigators. P. Schultz ('89) has explained the results of Pfitzner ('80) and Paulicki ('85) by saying that they studied sections which had been cut obliquely. However, Bruno ('06) found that in the frog multiplication of cells was not restricted to the lowest layer. In *Necturus*, although mitotic figures may have been more common in the germinative layer, there were numerous cases where unquestionably mitosis was taking place in the higher cell layers. The long axes of the spindles were always parallel to the body surface.

Intercellular bridges are not readily observed in preparations of normal tissue. Where, however, there has been some disturbance, such as a migration of cells to cover a wounded surface, they are easily distinguished. It is quite probable that they are present in all cases, but in the undisturbed epithelium the cells are so closely packed that the fine protoplasmic fibrils are hidden from view.

From the bases of the cells in the germinative layer fine tooth-like processes extend into the underlying connective tissue. A complete discussion of the relation of epidermis to dermis will be postponed till after the outer dermal layer has been described.

The preceding description has been limited entirely to the common elements which form any ordinary stratified epithelium. Besides these, many specialized structures are present. Large highly developed unicellular glands, shaped somewhat like Indian clubs, are found at frequent intervals over almost the entire body. These cells rest on the upper dermal layer and extend far up into the epidermis, displacing the ordinary epithelial cells. Goblet cells, highly branched black chromatophores and both pigmented and non-pigmented wandering cells are seen in greater or less abundance in many regions. Each of these special elements will receive consideration below.

B. Cuticular layer

The form of the cells comprising the cuticular layer is highly variable. The cells may be cylindrical, cubical, or even lens-shaped. The cell limits were easily recognized in all preparations. No mitosis was noted, but in a study of the 'casts' of this layer, double nuclei were noticed within single cells. It was impossible to tell whether they represented two separate nuclei or whether the appearance was due to a deep circular constriction. If one wished, this condition could be interpreted as evidence of amitosis. Schuberg ('92) described in the horny layer in the toad, binuclear and trinuclear conditions, the result, as he believed, of direct division.

1. *Cuticular margin.* a. In other Amphibia. The special feature of the upper layer of epidermal cells is the peculiarly differentiated outer margin, which, when well developed, is quite resistant to ordinary stains and shows very fine vertical striations. This striated zone has been repeatedly described and under many different names; many varied theories have also been advanced to account for the vertical markings. 'Cuticula,' 'pseudocuticula,' 'gestreifter Cuticularsaum,' 'poröser Cuticularsaum,' 'Randsaum,' 'Basalsaum,' 'Stäbchensaum,' 'eine von Porenkanälen durchsetzte Membran,' 'Deckplatte' and 'plateau striée,' are some of the more descriptive terms which have been applied to this structure by early writers.

The literature on this subject has been well reviewed by Studnička ('98) and O. Schultze ('07). F. E. Schulze ('67) speaks of 'durchsetzender Poren.' In his later works ('69, '88, and '96), however, he changes his interpretation and regards the margin as having a 'lamellösvacuolizierten' or 'gitternetzartigen' structure. He figures ('69, Taf. 18, Fig. 21-24) oval and round bodies contained in the vacuoles, suggesting a connection with some secretory activity of the upper layer of cells.

Leydig ('79), in his work on *Pleurodeles waltlii*, interprets the striations as due to thread-like protoplasmic structures lying in 'Röhrchen' of the cuticula. Wolff ('89, *Triton and Salamandra atra*) distinguishes two parts in the outer edge of the cells; one, a clear outer homogeneous layer, the 'cuticula,' and the other, a wider striated zone, the 'pseudocuticula.'

Cohn ('95, *axolotl*) identifies the striations as fine protoplasmic threads with the interfilar spaces filled with some dense, more resistant substance. In many cases, according to his description, no definite striations are present and the margin appears as an irregularly arranged protoplasmic network.

Studnička, in 1898, when discussing the appearance of the cuticula in surface view, says that it "aus ihre ganze Dicke durchsetzenden Lamellen gebaut ist, die so miteinander verbunden sind, dass sie die Wände langer röhrenförmiger Vacuolen vorstellen." In salamander larvae Prowazek ('01) finds that the cuticular margin resembles quite closely the alveolar margin of many Protozoa. Schubotz ('06), while denying the presence of any secretory activity, such as suggested by F. E. Schulze, claims for the cuticular margin an alveolar structure which is but the expression of the alveolar structure of the protoplasm.

O. Schultze ('06, '07) upholds the idea of the alveolar nature of the cuticular margin and concludes ('07, p. 556) his comparison of the condition of the margin in the larvae of the Anura and in salamanders with the following statement: "Es unterliegt aber keinen Zweifel, dass wir es hier, ebenso wie bei Anuren, mit einem wabenartigen, aus nebeneinander gereihten röhrenförmigen Alveolen aufgebauten Saum zu tun haben." He finds also that this layer has somewhat of a secretory function and describes

numerous cases, especially in the younger larvae of *Pelobates fuscus* and *Rana esculenta*, where a definite granule is contained within each alveolus. This secretion, he points out, gives the usual mucin reactions with Delafield's haematoxylin, Mayer's mucicarmine, and Hoyer's thionin.

Nirenstein ('08) refers to an 'Alveolarsaum' on the epidermis of young salamander larvae.

b. In *Necturus*, according to Eycleshymer and Wilson ('10), the cuticular margin appears on an 11-mm. embryo at the time when the epidermis becomes two-layered and the large unicellular glands are differentiated. Unfortunately, I did not have access to any very young material, and my observations are therefore limited to adult animals and one 10-cm. larva that came into my possession accidentally.

In preparations made from adult animals all stages of cuticular formation are seen, from the completely differentiated condition to stages where no definite margin can be distinguished. When fully developed, the margin appears to be sharply limited from the protoplasm of the cell body, and in this stage the striae extend completely across the margin. In intermediate stages the striae are visible only at the outer edge. In preparations cut perpendicularly to the surface and stained in the ordinary manner, it is impossible to determine, even with an oil-immersion lens, the nature of the striae or their relation to the underlying cytoplasm. When seen in surface view, however, some light is thrown upon the problem (fig. 8). The ends of the cells appear to be marked off into small polygonal areas whose walls enclose a less dense substance (O. Schultze, '07, figs. 3, 9, 24). The whole appearance is suggestive of an alveolar condition with the alveoli greatly elongated and tube-like. If this be true, the striations seen in vertical section would then be identified as the cut walls of elongated, closely packed alveoli. In preparations stained with Weigert's resorcin-fuchsin the cuticular margin is colored a deep purple or black, but still shows the familiar striae, although they now appear as very fine light lines separating into rod-like portions a densely stained substance. If we accept Schultze's ('07) interpretation of this structure in salamander

larvae as being adequate to explain the condition in *Necturus*, then the light lines would represent sectioned walls of alveoli, and the homogeneous densely stained masses would be identified as a contained secretion.

When the margin is well defined, the stain is limited entirely to it; but, in other cases, relatively large, flat, black plates of irregular outline are seen distributed through the cytoplasm in the vicinity of the nuclei of the cuticular cells (fig. 15). In these cells, farther away from the nuclei and toward the outer surface, the stain is taken also by fine scattered granules, which grow more numerous toward the outer margin, and become arranged immediately below the surface in fairly definite, parallel rows. Later stages show these rows of granules merged together to form the small rod-like masses which are characteristic of the fully developed margin. The reactions to mucin stains reported by O. Schultze ('07) were not obtained in *Necturus*, but resorcin-fuchsin, which stains this region so intensely, also stains readily the contents of the mucous glands.

These observations indicate that the cuticular margin is, in part at least, a secretion product, but not an extracellular one. The material which is laid down within the margin is elaborated in the deeper lying parts of the cells and moves to its final position in the form of fine densely staining granules. While we have no conclusive evidence that the cuticular margin is composed of closely packed, elongated alveoli, still the appearance is quite suggestive of such a structure, and it is quite conceivable that the secretion granules do collect in the alveolar spaces and later coalesce to form the homogeneous masses described.

In the row of cells immediately below the 'covering-layer,' staining reactions very similar to those already described are obtained. Flat densely-stained plates are seen grouped around the nuclei, and in the ends of the cells nearest the cuticular layer the concentrations of fine, dark granules are very conspicuous. This condition suggests that here is a replacing layer, in which some preparation has already been made for the new duties which must be assumed when the outer layer is cast off.

2. *Molting.* Kneeland ('57) states that *Necturus* sheds its epidermis in the winter. Eycleshymer ('06 a) reports, from notes made by one of his students on February 9, 1897, as follows: "The epidermis as a thin layer appeared to have loosened from the entire surface of the body, appearing frosty-white with bubbles of air. The loosened epidermis was split along the mid-dorsal line, its free edges floating upward in ragged streamers. On the following day none of the epidermis remained excepting glove-like portions which were yet attached to the feet; these portions were not cast until two days later." Reese ('05) finds that *Cryptobranchus*, when kept in captivity, sheds a 'thin transparent cuticle.'

I have invariably found that animals which have been subjected to trying conditions in the laboratory cast a thin layer of epidermis soon after. Mr. Cole's description, quoted by Eycleshymer ('06 a), is an accurate account of the process, but the time of the year, under laboratory conditions at least, does not appear to have anything to do with the act, since molting can be induced in the same animal several times in close succession by removing it from the water and allowing it to dry in the air for a short time. Only the cells bearing the cuticular margin are cast. Sections prepared from portions shed show the lower edge of the cuticular layer to be irregular, with the bases of the cells at higher and lower levels as they were when in position on the animal. Preparatory to molting, therefore, there is no flattening or other change in the form of the cells of the outer layer nor any differentiation of the outer surfaces of the underlying cells such as has been described by Schuberg ('91 a) for the tree-frog.

C. Horny layer

An actual horny layer, such as is described for land-inhabiting Amphibia, is found only on the hands and feet of *Necturus*. All of the outer cells over the rest of the body bear on their edges the cuticular margin, typical of all permanently aquatic Amphibia and of the water-inhabiting stages of the terrestrial forms. The cornification involves several layers and the cells are much

flattened (fig. 11). The nuclei are present in all layers. In *Siredon pisciformis* a stratum corneum is developed on the tip of the snout as well as on the extremities of the appendages (Carrière, '85; Paulicki, '85).

In the 10-cm. larva studied, an interesting condition was noted on the toes. Here the cornification involved the ventral surface only, while the dorsal surface possessed a typical cuticular margin. Passing from the dorsal to the ventral surface, no sharp line of distinction between the two conditions could be observed. The cuticular cells became gradually more flattened and their margins correspondingly thinner, till a stage was reached where the cuticular structure had entirely disappeared and it was impossible to distinguish between the two types of cells.

D. Club cells

The 'club' cells, so conspicuous in many preparations on account of their number, size, and staining qualities, are the same as the 'Schleimzellen' first described by Leydig in salamander larvae and *Proteus*. They have since been reported as present in many other Amphibia and have usually been referred to as cells of Leydig. Pfitzner ('79, '80; salamander larvae) and Carrière ('85; *Siredon pisciformis*) have followed their early development. They arise by a metamorphosis of cells in the stratum mucosum and later multiply by indirect division. Mitoses in these cells have also been reported by Paulicki ('85), Cohn ('95), and Prowazek ('01).

1. *Morphology.* In *Necturus*, according to Eycleshymer and Wilson ('10), these large 'mucous' cells appear in the 11-mm. embryo at the time the epidermis becomes two-layered. In adult animals the cells attain a considerable size, having, when seen in perpendicular section, an area six to eight times larger than that of the ordinary epithelial cells. They are club-shaped with their small ends resting on the dermis. They occur at varying intervals through the epidermis, in many regions lying uniformly close together (fig. 14). The epithelial cells in their immediate neighborhood always have the appearance of being displaced under a considerable pressure. In many cases where

the club cells are closely packed, the ordinary cylindrical cells of the germinative layer, greatly distorted as if wedged in between their large neighbors, assume forms adapted to the space left.

The nuclei of the club cells are smaller than those of the surrounding epithelial cells, and are usually spherical, but in many instances they become lobed or deeply furrowed. The furrows (perhaps more accurately described as constrictions) are sometimes so pronounced as to give the appearance of a binuclear condition which might have arisen by amitosis. The nuclei almost always lie near the base of the cells. In several instances ovoid cells were observed which, although smaller than the club cells and having central nuclei, stained densely and presented a general appearance suggesting an early stage of development of the club cells.

The bodies of the club cells color intensely in all plasma stains, but in no cases were they found to take any basic stain. Cohn ('95), however, reports that in axolotl the contents of the Leydig cells stain in iron haematoxylin similarly to the secretion of the large granular glands. (The affinity of the granules of the large glands for this stain, which has been reported by several workers, has not been demonstrated in *Necturus*.) The contents vary in appearance, generally looking like a reticulum with a very fine granular secretion contained within its meshes. At other times the reticular condition does not appear, and the cells seem to be crowded with brightly stained secretion granules. In one series of sections, however, the reticular and coarse granular conditions were found in combination within single cells. The granules were larger than any seen elsewhere and lay in the meshes of the reticulum. There is some evidence, to be advanced later, which indicates that this may be the mature condition of the gland cell.

The cell wall is not always clearly defined, but often appears as a mere marginal condensation of the cytoplasm or secretion, perhaps due to the action of the fixing reagent. Many writers have attached great importance to the appearance of the margin, since in this region they hoped to obtain evidence to substantiate the idea that these gland cells produced a secretion which escaped

into the intercellular spaces. If this does occur one would expect to find some evidence of at least a temporary dissolution of the cell wall. In two cases mononuclear leucocytes were found within the bodies of the club cells. In one the invading cell had rounded up and appeared to be at rest; in the other (fig. 5) the leucocyte was surrounded by a clear zone and its nucleus was elongated as if the cell had been in an active state when killed. No evidences of degeneration were noted in either case, and the entry of the leucocytes into apparently healthy cells would seem to denote a dissolution of the cell membrane, at least locally. In view of certain facts which will be stated later, it is also significant that in both cases the wandering cell made its appearance at the top of the club cell.

Another feature of the club cells is their distribution. Leydig ('76 a) noted that they were absent from the edges of the tail fin of many urodeles. Both Carrière ('85) and Paulicki ('85) have described them as being absent from the snout and extremities of the appendages as well as from the dorsal and ventral edges of the tail.

In *Necturus* restrictions in distribution similar to those reported by Carrière and Paulicki are found, and over the body there is a tendency toward local grouping, which is quite evident in whole mounts of strips of skin viewed with low power. Accordingly, in histological preparations made from different regions the club cells may appear very plentiful or may be entirely wanting.

2. *Function.* Although these cells are large and easily seen and have been observed by many investigators, their function still remains to a large extent an unsolved problem. Many theories have been advanced, but none are satisfactory. Langerhans ('73) regarded them as early stages in the development of the so-called beaker-cells which, in the toad (P. Schultz, '89; Muhse, '09), aid in separating the horny molt from the underlying stratum of cells. Pfitzner ('79) believed the cells of Leydig produced a slimy secretion which escaped into the intercellular spaces and possessed the power of protecting the outer cell layers from injury due to contact with the water.

Cohn ('95) thought that the cells were of a poisonous nature, similar to that of the large granular glands, and that they did

not discharge, but passively protected the animal from its enemies by simply serving as reservoirs for a poison which is presumably irritating to mucous membranes. Prowazek ('01, p. 86) concludes that they must produce an intercellular secretion which makes its way gradually toward the outer margin and protects the skin from microbic and other injuries. Schuberg ('07 c), in his work on the corium of *Siredon pisciformis*, incidentally describes in a foot-note (p. 564) and figures (Taf. XXVII, Fig. 2) a Leydig cell discharging freely on the surface.

In *Necturus* little variation in the form or position of these cells was observed. They usually remained in contact with the outer layer of the dermis, although occasionally they were slightly separated from it. In a very few instances they were spherical and situated high in the epidermis.

In the series of sections already referred to (p. 501), in which the secretion was in the form of large granules, some evidences of discharge were obtained. Several cells were found which had apparently ruptured at the upper ends and their contents had reached the outside, by way of the intercellular spaces, pushing apart the epithelial cells. The secretion appeared in small mounds or was flattened on the surface of the epidermis, and the collapsed club cells, containing only nuclei and remnants of a reticular network, presented a striking contrast to the plump, well-filled cells situated on either side of them. In other places small masses of the secretion appeared in the intercellular spaces above the tops of the cells, possibly indicative of an early stage in the process of emptying in which the cell contents had not yet forced a path to the exterior. This, however, may represent the normal activity of these glands, perhaps significant in relation to molting, the breaking through to the surface being an accidental occurrence. The conditions noted in this isolated series may have been due to some mechanical disturbance of the tissue before fixation, yet no other parts of the preparations presented any evidence of distortion or crushing.

In view of the small number of observations made, it seems unwise to attempt to draw any very definite conclusion regarding the possible function of the club cells in *Necturus*, but their

wide distribution and relatively high state of development make it impossible for one to regard them as unimportant in the life of the animal. They may never discharge, but, 1) may serve as reservoirs for a poisonous secretion (Cohn, '95), or, 2) they may produce intercellular secretions of value in keeping the skin in a healthy condition (Pfitzner, '79; Prowazek, '01), or, 3) they may actively assist in molting, or, 4) they may discharge at the time of molting, producing a secretion which protects the naked cells of the replacing layer until a cuticular margin has been developed. Satisfactory evidence as to which of these views is correct is still wanting.

E. Goblet cells

Goblet cells were found only in the region of the mouth and within the gular fold. Kingsbury ('94) states: "The transition from the outer skin is gradual; the glands of the cutis and the cells of Leydig cease upon the outer side of the lip, while the first goblet cells appear within the line of teeth." I, however, have found many goblet cells outside of the line of teeth, that is, on the lips.

In highly developed cases (fig. 16) the goblet cell is almost egg-shaped and tapers at the top into a narrow neck, opening between the cuticular cells. Its body has a reticulated structure and stains lightly. A much flattened nucleus surrounded by a small portion of non-metamorphosed cytoplasm lies at the base of the cell.

In the gular fold the goblet cells are similar to those of the mouth. I have been able to find only one reference to a similar local development of goblet cells. For axolotl, Paulicki ('85) reports goblet cells on "der inneren Fläche des Kiemendeckels."

F. Pigment

1. *Distribution.* The epidermis is deeply pigmented on the dorsal surface of the animal. Here the density of the epidermal pigmentation is quite closely correlated with that of the pigmentation of the adjoining dermis. Very little pigment is found in

the epidermis on the ventral surface, and in most cases none at all is present, although there may be considerable pigment in the dermis. Pigment occurs in the ordinary epithelial cells (fig. 1), but it is also contained in specialized cells (figs. 1, 2, 3, 4). The cylindrical cells of the germinative layer never contain pigment granules, but in all the cell layers above, collections of black or brown granules are commonly seen and they are nearly always found round the pole of the nucleus which is directed toward the outer surface (fig. 1). In the cells of the cuticular layer the pigment lies between the nucleus and the cuticular margin, and is often present in this position when pigment is lacking in the deeper epithelial cells of the same region. There can be no relation between the granules of pigment and the cuticular granules already described as staining readily in resorcin-fuchsin, for the cuticular granules are found in the outer layer in all regions of the body, while pigment is often lacking in this layer, especially on the venter.

Meirowsky ('06), in his experimental studies on the formation of epidermal pigment under artificial light, found that the granules first appeared uniformly around the nucleus, but later, under the influence of continued illumination, they moved and assembled themselves around the pole of the nucleus nearest the source of light. These results indicate that the distal distribution of pigment about the nucleus in normal tissue is probably determined by light.

Lying in the intercellular spaces of the transitional layers are numerous highly branched chromatophores (figs. 1, 2, 12). They have small oval bodies consisting of the nucleus and a small zone of cytoplasm, which is usually obscured by the pigment. Cells of this type are seldom found in the germinative layer, but their processes often extend far down between the cylindrical cells.

Besides the highly branched forms, other cells, more rounded in appearance and resembling contracted chromatophores, occur. They lie in spherical spaces in the epidermis, and, since they seldom fill these spaces, they give a pitted appearance to the tissue as seen in perpendicular section. Their nuclei are often

slightly indented and usually eccentric. The abundant protoplasm stains but lightly and is strewn with pigment granules. In many places short processes, sometimes with, sometimes without pigment, extend from the cell body to the surface of the space, and frequently they can be traced for some distance into the intercellular spaces. In these cells occur all variations from a densely pigmented condition to one in which only a few scattered granules are found.

2. *Origin of pigment cells.* a. Literature. The question regarding the origin of pigment in the epidermis of vertebrates is one of deep interest, and many investigations of the subject have been carried out on Amphibia both by students of histology and of pathology. The problem resolves itself into one primarily of relationships. What relation exists between ordinary epithelial cells, epidermal chromatophores, wandering pigmented cells, true leucocytes, and dermal melanophores?

Some writers hold that the epidermis is of itself unable to elaborate any melanin and that the pigment present is of mesenchymal origin (Kölliker, '87; Ehrmann, '92, '96). Reinke ('06) suggests the possibility of pigment being deposited within the epidermal cells by other wandering cells. Negre ('06) and Borrel ('13) also report an invasion of the epidermis by pigment from the dermal melanophores.

Many others, however, regard the epidermis as capable of forming pigment (Jarisch, '92; Rabl, '94; Rosenstadt, '97; Pro-wazek, '01; Loeb and Strong, '04; Grund, '05; Winkler, '10 a; L. Loeb, '11; Hooker, '14 c, '15), but they are not in agreement as to the extent to which this activity may be carried on by the epithelial cells. Some regard only the pigment in the ordinary cells as of epidermal origin, believing the specialized cells to have migrated into the epidermis (Rabl, '94; Eycleshymer, '06; Hooker '15). A few consider the epidermal chromatophores to be metamorphosed epithelial cells which have differentiated in situ (Jarisch, '92; Grund, '05; Winkler, '10 a).

According to Rabl ('94), all of the special pigmented cells in the epidermis are modified leucocytes. The nuclear and cytoplasmic fragments in their bodies are fragments of ingested red

blood corpuscles from whose haemoglobin the pigment is derived. The pigmentation may occur early, before the cells have reached the epidermis, or later, after they have come to lie within it.

Hooker ('14 c) cultured in plasma epidermal cells from frog embryos 3 to 4 mm. long. Pigment appeared in many cells which then migrated down into the culture and assumed a stellate form by sending out pseudopodia. He states: "Whether these cells remain as permanent pigment cells of the adult frog epidermis is uncertain and even questionable." Later ('15) he concludes that this pigment, elaborated by the embryonic epidermal cells, may exist for a time, but gradually disappears and the melanophoric cells found in the epidermis of older frog larvae are certainly mesodermic in origin.

Eycleshymer ('06 b) studied the differentiation of the epidermal chromatophores in the living larvae of *Necturus*. He observed them under the higher powers of a binocular microscope and was able to follow their movements accurately. He finds two kinds of chromatophores. "One is but slightly branched, taking on in general a pyramidal form. The other is highly branched, taking on a mossy appearance. The former becomes pigmented in situ within the epidermis. They may be mesenchymal cells which have wandered into the epidermis before becoming pigmented or they may be modified epithelial cells. The second type is derived from the mesenchymal cells which wander into the epidermis after becoming pigmented."

b. Conditions in normal tissue. In the adult animals I found no difficulty in recognizing the two types of pigmented cells described by Eycleshymer, but frequently intermediate conditions occur which make it appear probable that these cells have an identical origin. The pyramidal cells, or wandering cells of other writers, often contain little pigment and they may be completely rounded or possess distinct processes. But the most striking feature of many is the presence of large clumps of extraneous protoplasmic or nuclear material within their cell bodies, indicating phagocytosis (figs. 3, 4). This, coupled with the entire absence of pigment in the pyramidal cells of the white ventral region of the body, suggests some relationship between

them and the giant leucocytes reported by Kingsbury ('94) in the epithelium of the mouth, stomach, and intestine of *Necturus*. According to him, in the digestive epithelium, as in the epidermis, these cells occur in spherical vacuoles and contain densely stained elements. He also found numerous small leucocytes present in the vacuoles along with the larger phagocytic cells, and states: "A study of these (small leucocytes) is very suggestive and would seem to indicate upon what food the large leucocytes have fed that they have grown so great."

In the epidermis of *Necturus* both normal mononuclear and polymorphonuclear leucocytes are present (Claypole, '93; Berry, '97) and are frequently found in the vacuoles along with the pigmented cells, but no evidence has been secured to indicate that they are devoured by their large neighbors. Whatever the source of the ingested material in the wandering cells, whether it comes from disintegrating leucocytes, degenerate epithelial cells or other tissue, its presence bespeaks a phagocytic activity. But melanin cannot be the product of the included materials, as Rabl ('94) suggested, because pigment is never present in the white ventral region, where cells containing similar ingested material are found. We must therefore conclude that either these cells possess the capacity, under proper stimuli, of elaborating their own melanin or they acquire pigment directly by engulfing pigmented tissue (Ogneff, '08).

In some places the pyramidal cells appear to discharge on the outer surface of the body. A typical example is shown in figure 3. Long processes, containing nuclear and cytoplasmic fragments, extend up from the cell body through the intercellular spaces to the exterior, and, at the point where they reach the surface, the epidermis usually shows a distinct pit-like depression. The nucleus and main portion of the cell apparently remain undisturbed within the epidermal vacuole and only the ingested substances escape from the epidermis into the water. This seems to be an unusual condition. No information was obtained as to its significance.

The varying shapes of the pyramidal cells within the vacuoles, together with their ability to perform phagocytic work, can

leave no doubt as to their amoeboid character. However, they do not always occupy vacuoles, but are often found migrating through the tissue. Preparations made from regions which had previously been injured show them, along with polymorphonuclear leucocytes, in the immediate vicinity of the blood clot, ingesting red corpuscles. Pigmented cells are also found among the epithelial cells of developing glands (fig. 19) and in the lumen of large granular glands (fig. 22) after these discharge (Nirenstein, '08). In the last position they, in company with many leucocytes, are engaged in phagocytosis. At other times they are seen in the loosely woven tissue of the dermis, and in a very few cases have been found partly in the outer compact layer and partly within the epidermis (Rabl, '94; Prowazek, '01). Only one case of mitosis was observed in these cells, the dividing cell containing considerable pigment as well as several nuclear fragments.

The foregoing observations on the pigmented cells appear to support Rabl's belief that the epidermal chromatophores have a leucocytic origin. They at least exhibit many of the characteristics of leucocytes and contain pigment only when they are in a pigmented region. In the white ventral tissue they never contain melanin, but bear a close resemblance to the large phagocytic cells described in the digestive epithelium. Still, the evidence is not conclusive.

Winkler (10 a; Triton and Salamandra) believes that the epidermal chromatophores arise by a differentiation in situ of ordinary epithelial cells, which elaborate pigment, become amoeboid, and send out long branching processes. He regards all of the pigmented cells of the epidermis as belonging to one class and does not recognize two types such as Eycleshymer ('06 a) has described in *Necturus*. Furthermore, according to Winkler, these metamorphosed epidermal cells may migrate downward and enter the dermis. Thus we have two diametrically opposed views (Rabl, '94; Winkler, '10 a) regarding the origin of chromatophores, and it does not seem possible to end the controversy by a study of fixed material alone, since the conditions found can be interpreted in either way. We must therefore rely on experimental evidence and direct observation of living material for a final settlement of the question.

In *Necturus* the problem is rather complicated, since it is difficult to decide whether or not we are dealing with but one type of pigment cell. Eycleshymer's observation, while not conclusive, seemed to indicate that the cells have different origins, but, as he remarks, they may be the same kind of cells, merely acquiring pigment in different regions of the body. In my examination of several hundreds of slides I have found many conditions intermediate between the pyramidal cells and the more highly branched forms, suggesting that the latter were but extreme expansion phases of the former. In one case also (fig. 2) a large mass of cytoplasmic material was observed within the body of a cell which would ordinarily be regarded as a well-defined epidermal chromatophore. The facts presented suggest that we are dealing with but one kind of cell which may at different times be completely rounded, slightly expanded or very highly branched.

c. Conditions in regenerating skin. The adult *Necturus* possesses but small capacity for regeneration and the process is very slow. Where pieces of tissue were removed from the thin edge of the tail, regeneration proceeded gradually, but the gap was never entirely filled. Sections of this new tissue, made eight days after the operation, showed that the epithelium had moved outward over the wound, carrying with it chromatophores and ordinary pigmented epithelial cells. The chromatophores were usually without long processes and typical highly branched cells did not usually appear until some time later.

In regeneration the connective tissue lags far behind the epidermis, and in several cases eight weeks after the operation no dermal chromatophores had been developed. Occasionally I found small cells bearing pigment granules in the newly formed connective tissue. They may be leucocytes or developing chromatophores. In the epidermis, however, chromatophores were very plentiful. Two were found in mitosis in regions where rapid proliferation of ordinary epithelial cells was taking place. Both possessed long processes.

Pieces of skin (3 x 5 mm.) were removed also from the side of the tail for use in transplantation experiments. Four months

after the removal of the tissue, the tail of the living animal was examined under a high magnification, and it was found that the regenerated epidermis had become completely pigmented, both rounded and extremely branched cells being present, but no dermal chromatophores had yet made their appearance. These observations confirm the results obtained by Loeb and Strong ('04) in their studies of regeneration of the skin of the frog, and it is evident that the chromatophores of the regenerating epidermis cannot come from those of the dermis, since the former is completely pigmented long before the latter has acquired any melanophores.

d. Evidence from transplantation experiments. This method of investigation has been employed by other workers on pigmentation (Loeb, '97; Winkler, '10 a). Unfortunately, I did not begin my experiments early enough to secure conclusive results, but even in their incomplete condition they present several features of interest.

Rectangular pieces of pigmented skin, 3 to 5 mm. broad and 6 to 7 mm. long, were removed from the middle region of the side of the tail and patches of white skin of the same size from the venter were carefully fitted into the exposed places. In one case pigmented tissue was also transplanted to the white ventral region. Good unions were secured and in eight to ten days an abundant supply of blood was usually found in the grafted tissue. Changes in pigmentation occurred very slowly, and two months had passed before any definite evidence of the presence of pigment in the white tissue was obtained. At the end of four months the pigmentation had extended inward from the edge of the patches an average distance of $1\frac{1}{2}$ mm., but in no case was the entire tissue pigmented. In the regenerating tissue, it will be remembered, complete epidermal pigmentation had been accomplished within four months.

In the fifth month one of the white grafts, together with a narrow margin of the normal tail tissue, was dissected off and fixed. One half was imbedded and sectioned; the other cleared and mounted whole. The sectioned material showed that the pigment within the originally white patch was confined entirely

to the epidermis. No change whatever had taken place in the chromatophores of the dermis of the tissue surrounding the graft, although good connections had been established in this stratum. There was, however, an abundance of leucocytes in the dermis of the transplanted skin, and near its margins numerous small cells containing pigment were seen. It was not possible to decide whether the pigment in the epidermis was due to an immigration of chromatophores or whether it had developed in situ owing to some influence which was exerted by the surrounding pigmented tissue. The half which had been mounted whole gave a clear idea of the distribution of the chromatophores in the white patch. They were especially numerous near the margins, and cells without processes predominated. Nearer the center of the patch the cells were usually less densely pigmented. This condition argues against the idea of an immigration of chromatophores, since, if pigmentation occurred in that way, we should expect to find chromatophores of equal density in all parts of the newly pigmented region of the graft. Of course, this objection could be met by assuming that the less heavily laden pigment cells were more active than the dense chromatophores. Two other patches, examined on the living animals, also showed the cells with the least pigment to be nearest the center. The highly branched types were present and most of them were near the edges. In the fixed material many were greatly elongated and showed a definite orientation radially from the center of the patch.

The dark graft on the white venter was observed closely. For a long time after the operation no change in it was detected, but at the end of ten weeks a small light area was noted near its center. This area steadily increased in size and, at the time observations were discontinued, appeared as a large irregular region extending in places almost to the margins of the patch. When examined under a low power of the microscope it was seen that the light appearance was due to the absence of the dermal chromatophores. The epidermal chromatophores, however, were still present and a few could be seen scattered through the white tissue in the immediate vicinity of the transplanted pigmented portion.

e. Conclusion. According to Ehrmann ('92, '96), all chromatophores are differentiated from ordinary connective-tissue cells (melanoblasts), and some of them secondarily pass into the epidermis. The evidence furnished by these studies of *Necturus* gives no support to this hypothesis. In regenerating tissue the chromatophores are abundant in the epidermis before they develop in the dermis. Further, a study of the skin under normal conditions inclines one to favor Rabl's theory of the leucocytic origin of the pigmented cells, while their behavior in regeneration and in transplantation experiments strongly suggests a relation to ordinary epithelial cells.

At present, it is generally accepted that epidermal cells are capable of producing pigment. It is also known that in regeneration they become amoeboid temporarily and migrate considerable distances. In the formation of the large alveolar glands, epidermal cells may even move down into the dermis. Moreover, it has been found by L. Löb ('02) that epithelial cells in regenerating mammalian skin do actually take up blood corpuscles and other solid particles. Hence, it would appear possible that the specialized pigment cells found in the epidermis are not modified leucocytes, but deceptively similar epithelial cells which have become permanently amoeboid and often heavily pigmented, with the capacity of performing phagocytic duty.

DERMIS

A. Layers

1. *In other Amphibia.* The dermis exhibits in all Amphibia a division into several layers, due to the arrangement of the bundles of connective tissue. In most urodeles (Bugnion, '73; Paulicki, '85; Schuberg, '03; Esterly, '04; Schuberg, '07 c) it is divided into three layers—the outer compact, the intermediate spongy, and the inner compact. Also in the Anura three layers have been described, but the three regions designated do not always correspond with those of the Urodela. However, Stieda ('65), P. Schultz ('89), Weiss ('99), Grönberg und Klinckowström ('94), and Schuberg ('07 a, b, c, '08) have described three layers

comparable to those of the urodeles, an upper homogeneous layer, an intermediate and an inner dense layer. But Leydig ('67), Gaupp ('04), Muhse ('09), and Shipley and Wislocki ('15) have described the dermis of the Anura as divided into an outer loose, an intermediate compact, and an inner loose layer. The outer loose zone lies immediately beneath the epidermis. The outer homogeneous stratum, referred to by Stieda ('65) and others, they regard as the limiting surface of the outer loose layer. "Fibers from the outer loose layer terminate on the side toward the epidermis in fine branches. This gives the appearance of a very thin homogeneous stratum, which for the most part follows intimately the lower border of the epidermis" (Muhse, '09; p. 330). The inner loose layer, which they include in their divisions, is not a true part of the dermis, but represents a highly developed subcutaneous tissue, the 'tela subcutanea' (Gaupp, '04).

2. *In Necturus*. Although the subcutaneous tissue cannot be properly regarded as a part of the dermis, still it is so closely connected to it, that a brief description seems desirable. The thickness of this layer is variable. In many places the corium appears to rest almost immediately upon the muscles (fig. 6); in other places the subcutaneous layer is highly developed (fig. 12) and contains lymph spaces, blood-vessels, and the nerves which supply the skin. It is usually composed of fine, loosely arranged fibers, with an abundance of connective-tissue cells, but often the fibers are collected into large bundles which run for the most part parallel to the body surface. However, at frequent intervals (fig. 12, *tis. co'nt'*), they turn and pass perpendicularly through the inner compact layer into the intermediate spongy region, where they spread out and become interwoven with the bundles found there.

The inner layer (figs. 6, 12, *drm.'*), while in many cases not the thickest region, is by far the most dense. So closely felted are the fibers that with ordinary stains the region has the appearance of a homogeneous mass, and this effect is heightened by the scarcity of cellular elements. When stained by Mallory's method or in van Gieson's mixture the arrangement of the fibers becomes

quite evident. They are closely associated and usually occur in successive horizontal sheets, the fibers of adjoining layers running approximately at right angles to each other, so that in sections perpendicular to the surface cut ends and longitudinal views of fibers are seen in fairly regular alternation (figs. 6, 12). Some regions show these lamellae slightly interwoven. At certain points bundles from the compact horizontal sheets turn both outward and inward to join the vertical strands which pass upward from the subcutaneous region. Upright bundles are usually accompanied by blood-vessels and nerves going to the outer layers.

The middle layer (*drm.*') is the most variable of the three, both in thickness and in the arrangement of its elements. In this intermediate region, composed of loosely woven bundles of connective tissue, the majority of the glands are imbedded. In fact, this layer seems to have been differentiated chiefly to provide accommodation for these glandular structures, which grow in from the epidermis and attain such enormous sizes (Gaupp, '04; Schuberg, '03, '07, '08). In contrast with the layers above and below it, this region contains many connective-tissue cells, resembling in this the inner subcutaneous tissue. This stratum also receives bundles of fibers from both compact layers, but in regions where it is greatly thickened they cannot be traced very far. In regions where the glands are small and the intermediate layer is accordingly reduced, as on the side of the tail, bundles can be traced from their origin in the inner layer up over the bodies of the glands.

Above this open spongy region there appears in sections a narrow, well-defined, clear zone, the outer compact layer (*drm*'). This region in other Amphibia, on account of its close relation to the epidermis and its clear appearance, has often been mistaken for a basal membrane, especially by those who have worked on larval forms (Phisalix, '00 a; Eycleshymer and Wilson, '10). But Bugnion ('73), Paulicki ('85), and Schuberg ('03, '07, '08) have recognized that this layer is composed of connective-tissue fibers. In *Necturus* the composition of this region is quite readily determined. It is made up of sheets of fibers arranged

at right angles to each other as in the inner compact region. The layers here are, to be sure, much more delicate, but the arrangement is the same, and at frequent intervals bundles of connective tissue turn down and enter the middle layer.

A peculiar feature of the outer compact stratum is the manner of its union with the epidermis. On the bases of the cylindrical cells of the germinative layer, there are fine tooth-like projections, which extend into the dermis and blend with the connective-tissue fibrils. In other places large triangular projections of the epithelial cells are to be noted extending down into the outer compact layer, especially in places where perpendicular bundles turn down into the middle layer. In some cases these projections appeared to be united with the processes of connective-tissue cells, but it was difficult to demonstrate actual continuity.

In the vicinity of the edges of the tail the intermediate region disappears (fig. 17). The two compact layers come to lie quite close together with only pigment cells and blood-vessels in the position usually occupied by the spongy stratum. Perpendicular bundles which turn down from the outer layer often extend completely across the inner compact region and are lost to view in the loose tissue below.

As already stated, the glands on the body are usually imbedded in the loose intermediate zone, but occasionally a few are seen below the inner compact layer, lying in the subcutaneous tissue and next to the body muscles. In the edges of the tail no skeletal muscles are present, the entire space between the integument of the opposite sides being taken up by the subcutaneous connective tissue. In this region practically all of the glands are imbedded in the loose tissue between the inner compact layers of either side. In a few instances small glands were seen in the position which would have been occupied by the middle stratum if present.

B. Pigment

In the more heavily pigmented regions of the body the chromatophores are arranged to form continuous sheets beneath both the inner and outer compact layers. In addition they are

usually scattered irregularly through the whole of the middle layer (fig. 12). On the sides of the body and on the margins of the venter the pigment is limited to a layer immediately below the outer compact stratum (fig. 6). Both melanophores and xanthophores are present, but in fixed and stained material only the melanophores can be seen. However, in fresh material examined in glycerin or normal salt solution the xanthophores are clearly distinguished. They appear as large, horizontally flattened, branching cells closely packed with bright golden-yellow globules. The xanthophores are seldom situated deep in the skin, but usually lie at the level of the outermost layer of melanophores.

C. Blood supply

The skin has an abundant supply of blood, especially in the tail. Large blood-vessels are present in the subcutaneous tissue, and they probably form a horizontal plexus at that level. From there perpendicular branches pass outward through the compact layer into the middle region. In this position some break up to form fine capillary networks about the bodies of the glands, while others continue toward the surface and form a second capillary plexus just beneath the outer compact zone of the dermis. No blood-vessels, however, extend into this stratum.

D. Elastic tissue

Tonkoff ('00; Rana) was the first investigator to employ modern staining methods in the study of the distribution of elastic tissue in the amphibian integument. Schuberg ('03) gave a minute description of the arrangement of fibers in axolotl, and his results were confirmed in general by Esterly ('04) for *Plethodon*. In later works Schuberg ('07 c, '08) described the relation of elastic and connective tissue for many other Urodela and several Anura.

For a study of the distribution of these elements in *Necturus*, tissue was sectioned horizontally as well as perpendicularly and stained in Weigert's resorcin-fuchsin with van Gieson's mixture

as a counter stain. This method left the elastic tissue black or purple and the connective tissue red. (The elastic fibers are shown as black lines in figure 6.)

The elastic fibers are most numerous in the subcutaneous tissue, where they cross each other in all directions, forming an irregular network. From this region fibers extend down to the skeletal muscles and up through the inner compact layer in company with the perpendicular bundles of connective tissue already described. In fact, all through the dermis the elastic fibers parallel the connective-tissue bundles in their arrangement. Besides the fibers which accompany the upright bundles, numerous fibers are present in the horizontal layers of the inner stratum. In perpendicular sections of the skin, they are seen in longitudinal view and in cross-section, running in the same general direction as the connective-tissue fibers which make up the successive layers of this region. Frequently fibers may be seen to pass from one layer to the other. Sometimes they turn up into the middle region or down toward the subcutaneous tissue. Horizontal elastic fibers have never been found in the compact layer of the dermis of the *Anura*, but they have been described in *Proteus* and *Salamandra* by Schuberg ('07 c, '08).

In the intermediate spongy layer the fibers can be easily traced. They branch frequently, vary considerably in size, and all run in the same general direction, namely, from the inner dermis toward the epidermis. In the region of the glands, especially those of the granular type, they are more highly developed and are easily followed in sections which pass through the glands tangentially. They do not end beneath the gland, as stated by Schuberg ('03) for axolotl, but pass up around it, forming an open meshwork on its outer surface (Esterly, '04).

The elastic fibers grow gradually finer as they approach the outer dermal layer, and usually end before it is reached, a condition noted by several other workers (Tonkoff, '00; Schuberg, '03; Esterly, '04). In *Proteus*, however, Schuberg found that the elastic fibers extended completely to the epidermis. In *Necturus* I was seldom able to trace any beyond the outer layer of dermal pigment. Still occasionally along perpendicular

bundles very fine fibers could be seen extending into the outer layer, but they never quite reached the lower surface of the epidermis.

CHANGES IN COLORATION

A. Introduction

Necturus possesses the ability, common to most Amphibia, of changing its color through its control of the black chromatophores. Eycleshymer ('06 a) suspected this, but did not demonstrate it. Later ('14), in his work on *Necturus* larvae, he found that twenty-four hours after decapitation the black chromatophores contracted, some partially and others completely. Reese ('06), Pearse ('10), and Sayle ('16) have given accounts of the responses of *Necturus* to light, but they do not make any mention of color changes.

Only the dermal melanophores are concerned in the color changes. Those of the epidermis appear not to respond, but under any given condition some may be rounded, while others are branched. Laurens ('15) found that in *Amblystoma* epidermal chromatophores are more irregular and variable in their responses than the dermal pigment cells. Hooker ('14 a) did not find any correlation in response between the dermal and epidermal chromatophores of tadpoles. The xanthophores in *Necturus*, as far as could be ascertained, remain expanded under all conditions.

On account of the great variability in pigmentation, color changes are easily overlooked. A densely pigmented animal with contracted melanophores is darker than a light colored animal with expanded pigment cells, but under the microscope the individual cells can be observed, and no mistake as to whether they are contracted or expanded is possible. Changes in color, due to contraction of the melanophores, become apparent, first on the legs, and the venter, if pigmented, will also be found to have become much paler. On the sides and dorsal surface of the body the changes are not so noticeable. In very dark individuals the contraction of the melanophores produces practically no change in coloration, but sometimes a gray effect is noted on

the legs and at the bases of the gills. In the majority of animals, however, the yellow pigment, which is obscured by the black, becomes more apparent, and the black mottling, if present, is consequently emphasized. In the following discussion, when melanophores or pigment cells are spoken of, it is the black dermal chromatophore that is always referred to, unless otherwise stated.

B. Effects of light and background

1. *In other Amphibia.* Light has been shown to have an effect on the melanophores of various animals. Some of the earliest observations were made on *Rana esculenta* and *Hyla arborea* by von Wittich ('54), who found that in bright lights these animals were citron-yellow and in the dark, grass-green. His results were confirmed by Hering und Hoyer ('69), Dutartre ('90), Steinach ('91), and many others. Flemming ('97 a, '97 b) observed that larvae of *Salamandra maculosa* were light colored in bright light and dark when left in darkness. In general in the Amphibia bright illumination has been found to cause a contraction of the melanophores.

Rogers ('06) noted in *Diemyctylus viridescens* that, if the temperature of the water were kept constant and the intensity of the light increased, the color of the animal became correspondingly lighter. Babak ('10, '12, '13) found that axolotl larvae placed in the light were pale and those placed in darkness were dark. Hooker ('12) states that, in general, the warmer, dryer, and more highly illuminated the frog (*Rana fusca*) is, the lighter in color it will become. Pernitsch ('13) confirmed Babak's observations on axolotl larvae. With the larvae of *Amblystoma punctatum* and *opacum* similar results have been obtained by Laurens ('14, '15, '16), who found that animals kept in the light on an indifferent background are pale, while those kept in the dark are dark. But larvae placed in a bright light on a black background show a maximally expanded condition of all the melanophores. Furthermore, animals which had been kept in either darkness or light for a considerable time

(three to five days) exhibited what Laurens terms 'secondary' color reactions, which are directly opposite to those already outlined.

All the results that have been briefly reviewed show that bright illumination causes a contraction of the melanophores, although this effect may be offset by the color of the environment. However, Hooker ('14 a), in his experiments on the tadpoles of *Rana pipiens*, found that the dermal melanophores responded oppositely to those of the adult frog, expanding in the light and contracting in the dark. The color of the background had no effect on the response, and continued exposure to darkness produced a 'secondary' reaction of expansion of the melanophores.

2. *In Necturus*. *Necturus* shows responses to light similar to those noted by Hooker ('14 a) in the frog tadpole. In bright light on a white, black, or indifferent background the melanophores are always expanded. Animals placed in cool water in a photographic dark-room gradually become lighter in color, and a maximum response is obtained in about three hours. On their return to the light they regain their normal dark appearance in practically the same time. In bright light the color of the background has no effect on the nature of the response. But in *Amblystoma*, as has already been noted, a black background causes a complete expansion of the melanophores, which normally, on an indifferent or white background, would be contracted. The chromatophores of *Necturus* in dim illumination, as in the late afternoon, are usually partially contracted, and under these conditions animals on a white background are lighter than those in a black or gray environment. Hence we may conclude that strong light is a more effective stimulus than the color of the background, and when both are acting at the same time the environment is unable to produce any noticeable effect. Only when the light is greatly reduced, as in twilight, do we find the white background able to modify the response. No 'secondary' responses were obtained, although in many cases animals were left in the dark-room from ten to fourteen days.

C. Effects of temperature

1. *In other Amphibia.* The melanophores of various Amphibia have been shown to be affected by changes in temperature. Usually high temperatures cause a contraction of the pigment cells, while low temperatures favor an expansion. Most of the experiments with temperatures have been carried out in connection with light.

Early observers noted that frogs almost invariably become dark when kept at low temperatures (von Wittich, '54; Hering und Hoyer, '69; Knauthe, '91; Biedermann, '92; Ehrmann, '92 b). For *Salamandra maculosa*, Fischel ('96) found that the temperature to which the animals were subjected was an important factor. He observed that larvae which developed in warm water were light and that if dark-colored larvae were placed in warm water they became pale. Flemming ('97 a) thought that light alone was the cause of the color changes, but later ('97 b) discovered that temperature also affected the coloration of salamanders. He obtained maximum contraction of the melanophores by placing larvae in the light in a white dish containing water warmed to 24°C. A low temperature, however, inhibits the effects of the background and illumination, leaving the chromatophores partially expanded. Rogers ('06) found that a low temperature causes *Diemyctylus* to become dark, while a high temperature has an opposite effect. In this animal light also brings about a contraction of the melanophores and darkness an expansion. The response to light is less pronounced than that obtained by a change in temperature and may be obscured by the latter factor. When a combination of high temperature (35° to 40°C.) and darkness is made, or when a low temperature (10°C.) is combined with bright light, no response is obtained and an 'ordinary' coloration results.

Hargitt ('12) found that, while a high temperature brought about a lightening of the coloration of tree-frogs, no definite effect was produced by a low temperature. Laurens ('15) observed that low temperature caused the melanophores of *Amblystoma* larvae to expand, high temperatures (above 38°C.)

to contract. When the larvae are placed in a cold room (4° to $12^{\circ}\text{C}.$) the melanophores are always completely expanded, whether the animals be kept in total darkness or brightly illuminated on white or indifferent background. Hence in *Amblystoma*, up to $12^{\circ}\text{C}.$, temperature is more effective than either light or background. The effects of high temperature are not so noticeable as those of low, and complete contraction is never obtained by warming the animals.

2. *In Necturus.* The effects of difference in temperature on the melanophores of *Necturus* are not very apparent. Animals kept in a bright light, whether on a white, indifferent, or black background, are always dark, irrespective of changes in temperature. In darkness at low temperatures (9° to $10^{\circ}\text{C}.$) maximum contraction of the melanophores always results. Above $10^{\circ}\text{C}.$ and up to $20^{\circ}\text{C}.$, the response does not appear to be greatly changed, but quite often at the upper limit of temperature the contraction is not complete. Temperatures above $20^{\circ}\text{C}.$ always appear to inhibit the contracting effects of darkness, and at $25^{\circ}\text{C}.$ the chromatophores usually remain completely expanded as in bright light. High temperatures ($25^{\circ}\text{C}.$ and over), therefore, are more effective stimuli than darkness, but, in the light, low temperatures do not appear to have any appreciable effect on coloration.

It has been shown that *Necturus* differs from all *Amphibia*, except the tadpoles of *Rana pipiens*, in its responses to illumination and darkness. In the majority of animals high temperatures bring about the same results as light, and low temperatures have the same effect as darkness. It is not surprising, then, to find in *Necturus* that high temperatures, similarly to light, cause an expansion of the melanophores, where in other animals a contraction is always observed. Hooker ('14 a) does not give any information regarding the effects of temperature on the coloration of tadpoles, and no writer has described for *Amphibia* an expansion of chromatophores due to high temperature. However, in certain fishes (von Frisch, '11 a, 11 b) a high temperature brings about an expansion of the melanophores.

D. Effects of adrenalin

Lieben ('06) found that, in the frog, injections of adrenalin into any of the veins, body cavity, or lymph spaces caused a contraction of the melanophores. *Necturus* shows a similar response to adrenalin. An intracoelomic injection (concentration, 1 in 200,000) produced a complete contraction of the melanophores in about three and a half hours. This condition persisted for almost twenty-four hours.

E. Mechanism of contraction

1. *In other Amphibia.* The mechanics of the movements of pigment cells is still a disputed question. Many workers claim that the chromatophores are actively moving amoeboid cells. Others believe they are fixed stellate cells within which the pigment moves. Recent observers are agreed, however, that the proximal and distal migrations of pigment occur along fixed paths. Among the early workers the constancy of form of the pigment cells was not appreciated. The majority of them regarded the chromatophores as amoeboid cells contracting and expanding in intercellular spaces without following any preformed definite paths. Hooker ('12, '14 b) and Holmes ('13, '14) believe that the melanophores are amoeboid and that contraction and expansion are brought about by pseudopodia, but, according to Hooker ('14 b), the cells have constant expansion-phase patterns, which are forced upon them by the preformed spaces in which they lie.

Biedermann ('92) took an intermediate position, and claimed that, while the processes shortened in contraction, they were never completely withdrawn. Winkler ('10 b) induced contraction and expansion of melanophores by electrical stimulation, and found that in general the pigment follows old paths, but in some instances it may extend into positions not previously noted. In contraction, he believed, the pigment-free processes shortened, but he was unable to follow the movement very far. According to Kahn und Lieben ('07), in contraction and expansion the pigment moves in preformed paths and the form of the cell is

unchanged. Spaeth also holds to the theory that the chromatophores are fixed stellate cells, and recently ('16) he has brought forth considerable evidence to prove that the melanophores of fishes and Amphibia are not connective-tissue cells, but modified smooth muscle cells in which the motor function is lost and a modified motility, migration of pigment granules, is developed.

2. In *Necturus*. In *Necturus*, owing to the thickness of the skin, it was not possible to observe the movement of pigment under high magnifications, as Kahn und Lieben ('07), Winkler ('10 b), Spaeth ('16), and others have done. In material fixed and mounted whole, cells in various stages of contraction were studied. In many cases practically all the pigment was massed within the cell body, but occasionally enough was left scattered through the tissue to outline faintly the extent of the cell in the fully expanded condition. In other places, isolated clumps of pigment were seen lying some distance from the contracted cells as if they had been left behind when the main mass moved proximally.

It does not seem possible to reconcile these conditions with Hooker's theory of amoeboid movement. If the pigment in contraction is carried proximally in the cytoplasm by pseudopodia, none could be left behind, unless it escaped from the cell processes as they were being withdrawn. The appearance would be more readily explained by Spaeth's hypothesis, that the 'contraction' of the melanophore consists of an aggregation of the disperse phase of the melanin granules. In such coagulation, it is evident that scattered granules might be left in the processes of the cells and, if minute obstructions were present, the proximal movement might be hindered locally and the granules would collect at these points to form small clumps of pigment. In sections of contracted melanophores, however, it was not possible to distinguish empty cell processes.

GLANDS

A. Introduction

The large glands of the skin of Amphibia have been favorite subjects of study for many years. Work has been done on animals of all three orders. The investigations on the Gymnophiona have been limited; the Urodela have been studied intensively, and, of the Anura, the families Ranidae and Bufonidae have received most attention. The number of kinds of glands has been, and still is, a disputed question. Most writers have described at least two kinds. These have been variously designated as large, granular, poison, or contractile, and small, clear, mucous, or non-contractile, respectively. But a few investigators hold that there is only one kind of gland in the skin and that the various glands of other authors are but different stages in the development of it.

In several papers descriptions of mixed glands, partly of the mucous and partly of the granular type, have been given. There is much diversity of opinion regarding the significance of these double structures. Several workers regard the mixed gland as arising from a process of replacement of a granular gland by a mucous gland, but they are not agreed upon the ultimate fate of the replacing elements. Some believe that the mucous cells retain their original character and produce mucus during their entire period of activity, while others think it highly probable that they assume the function of the granular cells which they have replaced. A second explanation of the mixed condition has been given by those who contend that the granular glands in their development always pass through a mucous stage before reaching their final appearance and character. According to this view, mixed glands would represent incomplete stages in the transformation of mucous glands into granular glands. However, the possibility of a mucous cell metamorphosing into a poison cell has been vigorously denied by many, who claim that a mucous cell is always a mucous cell and a granular cell always a granular cell. Hence, even among investigators believing in the existence of two kinds of glands, we have a diversity of

opinion regarding the relation between the mucous and granular types. Some, although they admit the possibility of replacement of one type by the other, hold that each kind of gland has a separate development and that the cells never become transformed into anything different from what they originally were. Others, while they recognized two functional types of glands as being present in the skin, nevertheless believe that the granular cells have a mucous stage and later become differentiated toward a special function.

According to those who contend that there is but one kind of gland, the small or mucous glands are young stages of the large or granular ones. This view is not entirely unlike that expressed by those who hold that the granular glands are differentiated from those of the mucous type.

In *Necturus* two groups of seemingly quite different glands are found. Some are large, well rounded, and completely filled with a dense granular secretion. Others are smaller and do not present the plump appearance of the larger glands. The secretion in these is fibrous or vesicular and stains lightly in ordinary preparations. There is nothing in the form or character of the smaller glands to indicate that they are young stages in the development of the granular type. Their general appearance would rather lead one to regard them as discharged granular glands. I have been unable, however, to find any genetic relation between the two types; on the contrary, there is every evidence that we are dealing with two distinct types which differ in development, in histological structure, in the character and staining reactions of their secretion, and in their physiological activities. Mixed glands are occasionally found. They represent stages in the replacement of the mucous glands by glands of the granular type. It also appears possible that a permanently mixed condition may exist. After expulsion of secretion the granular glands may regenerate, but the replacing epithelium is never mucous in character.

B. Evidence of the presence of two types of glands

1. *Literature.* The literature dealing with the skin glands, specifically or incidentally, is extensive, and good historical summaries have been presented by Gaupp ('04) and Nirenstein ('08). Nevertheless, a brief statement of the views held by the various investigators will be given at this point. That the glands are of two kinds is an opinion shared by the greater number of investigators, but the criteria for such a distinction are not always the same. Early classifications into clear versus granular, mucous versus poison, and non-contractile versus contractile have been based on differences in structure and in the secretion produced or on physiological activity.

The terms 'Schleimdrüsen' and 'Körnerdrüsen' were first used by Englemann ('72), and later writers who recognized the existence of two types of glands have for the most part adopted this terminology. Physiological studies and experiments prove clearly that the skin of many Amphibians secretes a poisonous substance (Phisalix, '89, '90, '97, '00 a; Boulenger, '92; Hubbard, '03; Nirenstein, '08; Abel and Macht, '11; Shipley and Wislocki, '15). Since the poison was generally isolated from the granular glands, these were often referred to as poison glands ('Giftdrüsen,' 'glandes à venin'). Other researches (Phisalix et Dehaut, '08; Phisalix, '08) have shown that the mucous as well as the granular secretion may possess poisonous properties. Ancel ('02), in his account of the development of the glands in terrestrial salamanders, uses the terms contractile and non-contractile to designate the two kinds of glands. This classification, however, has not come into common use, as many writers have described a muscular layer around both types. The designation as mucous and granular glands, suggested by Englemann ('72), appears to be open to the least objection, since practically all observers agree in their descriptions of the appearance of the secretion, while there is diversity of opinion regarding the poisonous character of the secretion and regarding contractility. Bruno ('04), in *Rana esculenta*, classifies the glands as holocrine and merocrine.

A few investigators have advanced evidence or expressed belief in one kind of gland. Bugnion ('73) found only one kind

of gland in the skin of *Proteus*, and this corresponds with the mucous type of other authors. His results were confirmed later by Leydig ('76 a) and Phisalix ('12). Also Calmels ('82, '83), Leydig ('92), Junius ('96), and Muhse ('09) describe one kind of gland and believe that the mature gland is of the granular type, regarding the so-called mucous glands as younger stages of it.

2. *Morphology.* a. Distribution, form, and position. Under this heading (a) are considered the mature glands, which are uniformly present in every preparation of integument. Over the general body surface of *Necturus* the glands, as in all other *Amphibia*, are of the simple alveolar type. Within the cloaca, large groups of long tubular glands, typical of all urodeles, are found. No glandular pads, such as have been described for tree-frogs and other *Anura*, are found on the hands and feet. The digits, unlike those of *Salamandra atra*, *Triton*, *Bufo variabilis*, and *Alytes obstetricans* (Gaupp, '04), are entirely free from glands of either kind. Also on the ventral surfaces of the hands and feet, glands are seldom found and none are present on the gill trunks or within the gular fold. Over the remaining parts of the body, however, both granular and mucous glands are found in more or less abundance. The glands in *Necturus* are never grouped and there is no tendency toward specialization in any region, except within the cloaca. Granular glands are largest and most plentiful on the dorsal surface of the body and in regions along the dorsal and ventral edges of the tail. On the venter, the mucous glands are more abundant than the granular and very large mucous glands are found around the margin of the cloacal opening. There are few alveolar glands on the arms and legs, and all are small.

As has already been stated, all the glands except those in the region of the dorsal and ventral edges of the tail lie in a spongy connective tissue, the intermediate layer of the dermis (figs. 6, 12). They consist of three parts: duct, collar ('intercalary region,' 'Drüsenhals,' 'Schaltstück'), and alveolus. The ducts of both mucous and granular glands pass perpendicularly through the outer layer of the dermis and the epidermis. The collar is

an accumulation of cells, often several layers thick, at the bottom of the duct, and marks the transition from the duct to the body of the gland. The bodies of the granular glands form large sacs extending, when seen in section perpendicular to the surface, completely across the spongy connective tissue, and the bottoms of many large glands depress the inner dermal layer. The gland sacs are usually somewhat ovoid, the perpendicular diameter (maximum $925\ \mu$) being much greater than the transverse (maximum $700\ \mu$).

Where the glands are not crowded the typical ovoid outline is realized, and in horizontal section they then appear more or less nearly circular. If, however, they are closely packed and affected by mutual pressure, they appear in vertical section somewhat rectangular in outline, and in horizontal sections present a variety of polygonal shapes. The general form of both glands is the same except that the mucous glands usually present a slightly irregular outline, while those of the granular type always appear completely rounded. The mucous glands, moreover, are not so deeply inbedded in the dermis and, in regions where the granular glands are large and closely associated, those of the mucous type are found lying between the necks of the large glands immediately below the thin outer dermal layer.

b. Histological structure. a) Ducts. The ducts of the granular glands are usually cylindrical, but when the glands are highly developed the lower parts of the ducts are usually distended by the secretion which is forced upward from the sacs (fig. 6). When this occurs the epidermis is depressed and appears in surface view as a pit into which the duct opens. The epidermis surrounding the ducts does not exhibit any great differentiation such as has been described for many Amphibia. The cells immediately around the duct are, to be sure, modified in form, but no peculiar structures or staining reactions have been observed. In many other salamanders 'funnel cells' and 'replacing cells' have been described (Nicoglu, '93; Ancel, '02; Esterly, '04), and Eberth ('69) speaks of a 'stoma cell' at the external opening of the duct in the frog skin. The cuticular margin, present on the outer layer of epidermal cells, is never found on the cells

lining the duct, which, in fact, seems to be nothing more than a large intercellular space extending from the body of the gland through to the outer surface of the epidermis.

The ducts of the mucous glands (fig. 12) appear as straight cylindrical openings piercing the epidermis. The cells lining the duct are usually definitely arranged with their long axes in planes at right angles to the long axis of the duct and encircling it. In perpendicular sections, therefore, the cells are seen in end view and appear smaller than the ordinary epithelial cells. Their nuclei also stain deeply. The transition from the body of the gland to the intercalary region and duct is sharper than in the granular glands.

b) Intercalary region. The collar or intercalary region is present in both kinds of glands in *Necturus*, but is more highly developed in the granular glands.

For the poison glands in *Triton*, Nicoglu ('93) and Heidenhain ('93 a, '93 b) have described the collar as a structure consisting of some four cells arranged in a ring around the base of the duct. Vollmer ('93) finds the 'Schaltstück' often absent in *Triton*, and Esterly ('04) states that in *Plethodon* it is not demonstrably present except in one or two questionable cases. Schultz ('89) has described a sphincter muscle composed of a band of muscle fibers running around the neck of the gland, and Phisalix ('00 a) refers to an 'orbicular' muscle in this same region. Arnold ('05) finds that there is sometimes an approach to a circular arrangement of cells at the neck of the gland, but there is nothing to indicate that they are of a muscular nature. Esterly ('04), however, describes both dilator and constrictor muscles about the mouths of the poison glands of *Plethodon*. These muscles are ectodermal and lie wholly within the epidermis. They have to do with the manipulation of the lumen of the duct during the expulsion of the secretion. Drasch ('94) described an epithelial plug which he believed served to restrain the contents of the gland and was forced out when the secretion was expelled. In *Necturus* the structure of the intercalary region is very similar to that of the glands of the toad (Muhse, '09). In sections parallel with the surface and cut through the collar, the inter-

calary cells are seen to be radially arranged about the duct, giving rise to the 'cog-wheel-like structure' of Muhse. The uppermost of these cells are in direct contact with cells of the germinative layer of the epidermis. In perpendicular section the region appears as several layers of cells overlapping each other tile-like on either side of the gland neck (figs. 6, 12, 13, 32). In the granular glands they form a firm region of attachment for the muscle fibers extending downward over the body of the gland. On the mucous glands the intercalary cells are usually less numerous, but always present. Very few authors have described a distinct collar for the mucous glands in other Amphibia.

c) Gland alveolus. The walls of the granular glands are composed of three layers. The outer, or tunica fibrosa, is made up of closely packed connective-tissue fibers, which interlace over the surface of the gland and are continuous with the fibers forming the bundles of the intermediate spongy layer of the dermis. In this region are found the highly developed elastic fibers already described and the nerves which supply the gland muscles and gland epithelium. Within the connective-tissue sheath lie the muscle fibers and next comes the epithelium of the gland. Several writers (Drasch, '94; Junius, '96; Bruno, '04; Reese, '05; Nordenskiöld, '05) report a tunica propria present in other Amphibia. This they regard as a structureless membrane, comparable to a basement membrane. According to Schultz ('89) and Weiss ('99), the glands are invested by a continuation of the upper 'Cutisraum,' which was pushed downward by the gland during its development. But Engelmann ('72), Gaupp ('04), Esterly ('04), and Nirenstein ('08) report a condition very similar to that in *Necturus*, where the gland is closely surrounded by connective tissue continuous with that of the middle dermal layer. Muhse ('09) and Shipley and Wislocki ('15) state that in the toad the wall of the gland sac consists of a homogeneous matrix. The muscle fibers are inbedded in the outer surface of this matrix and on its inner surface lie the epithelial cells.

The walls of the mucous glands in *Necturus* consist of but two layers, the connective-tissue sheath and the gland epithelium. The muscle layer is absent. No tunica propria or basement membrane is present. Arnold ('05) describes in the mucous glands of the frog a membrane, composed of very fine wavy fibers, which extends between the gland epithelium and the muscle layer. This membrane stains intensely in elastin stains. Nothing comparable to this was observed in *Necturus*.

d) Muscles of granular glands. Smooth muscles on glands were first demonstrated histologically by Hensche ('56), though earlier observers had noted movements of the living glands. Practically all writers since then have recognized the existence of muscles on the large granular glands. Reese ('05), however, in his brief description of the integument of *Cryptobranchus*, states that "The muscular layer sometimes described cannot be made out." Calmels ('83) and Seeck ('91) evidently mistook transverse sections of muscle fibers for epithelial cells. In *Bufo aqua* (Shipley and Wislocki, '15) the muscles are attached to the collar and run down over the gland body in a spiral manner. In *Bufo americanus* (Muhse, '09) the fibers are arranged meridionally, and it often requires several fibers to complete the circuit of the gland. There is, therefore, in this animal no definite arrangement of the muscle nuclei in any given region as Esterly ('04) found in *Plethodon*, where the nuclei of the contractile cells, contrary to the description of Nicoglu ('93) and Vollmer ('93) for *Triton*, "lie in the upper region of the glands just outside the uppermost gland cells, yet still well beneath the epidermis." Further, Esterly (p. 236) states "That those observers, who describe muscle nuclei on the periphery of the gland sacs, have mistaken connective-tissue nuclei for them, seems to me very probable."

Drasch ('95) found in the salamander, around the periphery of the large poison glands, a complete layer of smooth muscle fibers united by wide anastomoses into a syncytium, and Esterly ('04) and Muhse ('09) describe branching muscle fibers on the lower part of the gland. Several writers (Eberth, '69; Leydig, '76 a; Nicoglu, '93; Heidenhain, '93 a, '93 b; Drasch, '94; Ancel,

'02; Esterly, '04; Tarchetti, '04) state that the muscles at their upper ends are divided into fibrillae which pass into the epidermis and provide for the insertion of the fibers.

As in other Amphibia, the muscle fibers on the granular glands of *Necturus* are of the non-striated type, greatly elongated and spindle-shaped, with long and much flattened nuclei. They are arranged in a single continuous layer and lie in a meridional direction converging toward both poles. Flattened connective-tissue nuclei are often found around the bodies of the glands, but, with differential stains, such as Mallory's and van Gieson's, they are easily distinguished from the nuclei contained within the muscle. Sections cut parallel with the surface of the integument also show nuclei within the muscle fibers at many different levels, with no tendency to such grouping as is described by Esterly ('04) in *Plethodon*. I have followed many fibers through their whole extent and have found them to be without exception simple spindles, but in many cases it requires two or more fibers to reach from the upper to the lower pole of a gland.

The outer ends of the muscle fibers extend in between the cells of the intercalary region, which appears to form a fixed point for their insertion. This condition is very similar to that described for several toads (Nordenskiöld, '05; Muhse, '09; Shipley and Wislocki, '15), except that the collar in *Necturus* is never so highly developed. There is no evidence whatever that the fibers terminate within the epidermis. The muscles were traced in sections cut parallel with the body surface and their outer ends were found to be indistinguishably blended with the radiating cells of the intercalary region, which is of course continuous with the epithelial cells above. At their lower ends the muscles found on the upper portion of the glands are overlapped by the tapering ends of other fibers which lie lower on the gland sac.

e) Muscles of mucous glands? There is diversity of opinion regarding the presence of a muscular layer on the mucous glands. The absence of muscle fibers has been used by several writers as a character to distinguish them from the granular glands. Many investigators describe a muscular layer which is not continuous and is easily overlooked unless several sections are studied in

series (Ciaccio, '67; Szczesney, '67; Eberth, '69; Engelmann, '72; Drasch, '89; Schultz, '89; Grönberg und Klinckowström, '94; Junius, '96; Weiss, '99; Gaupp, '04; Bruno, '04; Nordenskiöld, '05; Arnold, '05). On the other hand, several others deny the presence of muscles on glands which are mucous in nature (Stieda, '65; Coghill, '99; Ancel, '02; Esterly, '04; Reese, '05; Phisalix, '10).

In *Necturus* there certainly are no muscles on the mucous glands. In perpendicular sections which are tangential to the surface of the sacs of mature glands, no longitudinally arranged cells can be found, and, furthermore, in sections cut parallel with the body surface no evidence of the existence of a muscular layer is obtained. If muscle fibers were present they would surely be demonstrated in the latter sections, since in these they would be seen in cross-section.

f) Epithelium and secretion of granular glands. A syncytial condition of the secreting epithelium has been described in many other Amphibia (Leydig, '76 a; Drasch, '94; Seeck, '91; Esterly, '04; Reese, '05; Arnold, '05; Nordenskiöld, '05; Nirenstein, '07; Muhse, '09; Shipley and Wislocki, '15). According to Heidenhain ('93 a) and Nicoglu ('93), in Triton the poison cells are sharply limited on all sides. They, however, describe 'überreife' conditions in which the cell limits are broken down and the granular secretion lies free in the lumen. Leydig ('67), Engelmann ('72), Seeck ('91; Rana, Triton, Salamandra), and Weiss ('99) find that the cell walls are obliterated only toward the center of the gland, while nearer the gland wall the cells are distinctly separated. Schultz ('89; Salamandra) and Junius ('96; Rana) describe a discontinuous epithelium for many granular glands.

In *Necturus*, within the muscular layer of the mature granular glands, are the remnants of the cells which once formed the secreting epithelium (figs. 6, 12). All traces of cell boundaries are gone and only naked nuclei are left lying at the periphery of the gland imbedded in the granular secretion. The nuclei are not evenly distributed. Over small areas they may be closely associated, in other places they may be entirely absent. Occasionally nuclei occur in the center of the gland sac, but they

usually lie at the outer surface close to the muscular layer. They are usually spherical or ovoid, but are sometimes greatly elongated and sometimes much flattened. They stain approximately the same as the nuclei of ordinary epidermal cells, and show an irregular chromatin network with occasional large chromatin masses. No nucleoli were observed. A more complete account of the nuclei will be given in the description of the histogenesis of the granular secretion.

The body of the mature gland in the resting condition is always found entirely full of the granular secretion, which, judging from the form of the gland, must be held under considerable pressure. When expelled the secretion appears on the surface of the skin in small heaps. It is gray and does not flow readily. Fresh secretion examined with the microscope in normal salt solution is seen to be a liquid crowded with innumerable globules which vary in diameter from 1 to 10 μ and are not always perfect spheres. They are easily distorted under pressure, and if crushed they break down into very fine granules. The liquid in which the globules are suspended is colorless and somewhat viscid.

The secretion is well preserved in 2.5 per cent formaldehyde or in a saturated solution of corrosive sublimate. The globules, when fixed, appear as large discrete granules, closely crowded within the gland. In other fixing fluids (Kleinenberg's, Gilson's, Flemmings's) the secretion presented an alveolar appearance with the alveoli filled with very fine granules. The disintegration of the large granules is without doubt due to the fixing agents. Arnold ('05) obtained much the same result with fixing fluids. But in the earlier stages of its elaboration, the granular secretion of *Necturus* is well preserved in all fixing fluids and always appears as distinct granules. Several other investigators have found difficulty in obtaining good preservation of the mature secretion, while they experienced no difficulty with the secretion in its early stages. In Triton, Nicoglu ('93) and Heidenhain ('93 a) find that the granules fix readily till they attain a diameter of from 3 to 4 μ . After they become larger their appearance when fixed is greatly changed.

In his description of the development of the poison granules in the glands of *Salamandra maculosa*, Nirenstein ('08) distinguishes two stages in the formation of the secretion. In the first stage the granules are 'albuminoid' in nature, coagulate readily in all fixing fluids and retain their spherical form. In the second stage the specific poison, salamandrin, is elaborated and the granules do not retain their form in fixing fluids. The secretion when mature is surrounded, he believes, by a semi-permeable membrane, and in fixing solutions the alkaloid poison diffuses out and the granule is thus destroyed.

In *Necturus* the changes in appearance between the immature and the completely elaborated secretion are not so striking as those described by Nirenstein, but the fact that the secretion in different stages of development does react differently to the same fixing fluids indicates clearly that there is a change in its chemical composition. In all its stages the granular secretion takes ordinary plasma stains very readily and the glands always have a decided color. The secretion mass is colored red or dark purple in Mallory's and yellow in van Gieson's stain. The granules are never colored in such basic stains as iron haematoxylin, Ehrlich's or Delafield's haematoxylin, thionin, or methylene blue. However, Heidenhain ('93 a), Nicoglu ('93), Esterly ('04), Arnold ('05), and Nirenstein ('08) find that the granules of this type of gland stain black in iron haematoxylin. No experiments were attempted to prove whether or not the granular secretion was poisonous.

g) Epithelium and secretion of the mucous gland. In contrast with the granular glands, which act as reservoirs for secretion, the mucous glands (figs. 6, 12) in the majority of preparations are seen to be actively discharging secretion onto the surface of the epidermis. In many Amphibia they have been described as consisting of a single layer of secreting cells surrounding a capacious lumen, but in *Necturus* the secretion occupies the entire body of the gland and the secreting epithelium is greatly reduced. Cell limits are seldom perceptible, and the small, irregularly shaped, and densely stained nuclei are scattered unevenly over the wall of the gland. No cytoplasm can be

distinguished in many cases, but in preparations stained only with a plasma stain a small quantity can often be distinguished in the immediate vicinity of the nuclei. The nuclei are smaller than those of the granular glands and are usually flattened against the gland wall, appearing angular in radial sections and round or oval in tangential sections of the gland. They invariably stain deeply in haematoxylin and retain their color even after the nuclei of the surrounding tissue have been almost completely decolorized. They show very little chromatin structure, but appear as homogeneous masses.

The fresh secretion is clear and slimy. In sections of fixed material it usually appears fibrous or vesicular. The fibers, when present, always lead toward the duct, giving one the impression of a viscid substance in motion (fig. 12). Besides differing so greatly from the granular secretion in general appearance, the clear secretion also reacts very differently to stains. The contents of the glands are stained blue by Mallory's connective-tissue method, and with van Gieson's method the secretion takes on a clear red or pink without a trace of the yellow invariably found in the granular glands. In iron haematoxylin the secretion is left practically unstained, but in either Delafield's or Ehrlich's haematoxylin it takes a decided blue color, especially if the stain is a little heavy and the sections are 'blued' in an ammoniacal solution. Hoyer ('90) and Nicoglu ('93) have used thionin as a specific stain for mucus and found that the glands were stained a red-violet. I have used thionin successfully in staining these glands, but was not able to retain the red-violet color when the sections were passed up through the alcohols and cleared preparatory to mounting in balsam. Preparations stained and examined in water, however, gave the typical color reaction, but when they were dehydrated and cleared, the red was entirely gone and the secretion was left a deep blue. Thionin has absolutely no effect on the granular secretion. Hubbard ('03) and Esterly ('04) were unable to obtain any color reaction in the mucous glands with thionin. Weigert's resorcin-fuchsin, a selective stain for elastic tissue, while it has no effect on the granular glands, always colors the mucous glands a deep purple.

or black. Esterly ('04) reports that, in *Plethodon*, Tänzlers orcein, another elastin stain, colors the cytoplasm of the mucous gland a deep brown.

The foregoing observations prove beyond a doubt that, at least in the mature stages we are dealing with, two distinct kinds of glands, granular and mucous, exist. The granular or poison glands are larger than the mucous glands and appear more distended. While there is a well-developed muscular layer enveloping the granular glands, none is ever found on those of the mucous type. The nuclei of the granular cells are large and rounded and stain as do the nuclei of ordinary epidermal cells. The nuclei of the mucous cells, on the other hand, are irregularly flattened and stain intensely in all basic stains. Further, the granular secretion is very readily colored in all plasma stains, but is untouched by nuclear stains. In contrast with this, the secretion of the mucous glands, while it stains well in Delafield's or Ehrlich's haematoxylin and in thionin or Weigert's resorcin-fushsin, is not affected by ordinary plasma stains. It is also never found in the form of granules.

3. *Development.* a. In other Amphibia. With one exception, Phisalix ('00 a-e, '03), all writers are agreed that the glands have an ectodermal origin and develop from the epidermis. Nicoglu ('93) and Heidenhain ('93) concluded, from their observations on the relation between the intercalary region of the glands and the epidermis in Triton, that the glands must be formed by an invagination from the malpighian layer. Maurer ('95) showed that in *Salamandra*, Triton, and the frog, the glands have this origin, appearing within the epidermis as a compact mass of round cells with an irregular arrangement. The cells multiply by indirect division and later push down into the dermis. Junius ('96) confirms for the frog Maurer's description of their development.

According to Phisalix, however, the glands of the salamander are of mesodermal origin. They develop from cells in the dermis which multiply mitotically and acquire a union with the epidermis. Anceel ('02) has followed closely the development of the glands in *Salamandra*, Triton, Alytes, Siren, and *Amphiuma*.

He concludes that the glands of batrachians have an ectodermal origin and that, although they come to lie within the dermis, they are never completely separated from the epidermis. Tarchetti ('04) also finds that in the regenerating tail of Triton the glands develop in this way, and Muhse ('09) states that in the adult toad new glands are continually being differentiated from the epidermis.

While there is a general agreement regarding the origin of the glands, there is considerable diversity of opinion regarding their later development and differentiation. Engelmann ('72), Seeck ('91), Schultz ('89), Heidenhain ('93 a), and Nicoglu ('93) hold that the mucous and poison glands have an entirely separate development and that there is no relation between mucous cells and poison cells. Ancel ('02), however, states: "Les petites et les grosses glandes de la Salamandre ont un développement absolument semblable. Les glandes à venin ou grosses glandes représentent des organes plus parfaitement différenciés que les petites glandes vers une adaptation fonctionnelle spéciale."

Fano ('03) is of the opinion that in Triton the mucous glands of other authors are but rejuvenating stages of the poison glands, and Esterly ('04) is inclined to explain the conditions in *Plethodon* in a similar way. In *Salamandra maculosa* Nirenstein ('08) finds that the poison glands are developed from three sources: indifferent young glands, mature slime glands, and small reserve glands ('Ersatzdrüsen' of Drasch, '94). Weiss ('08, '15) also holds that in frog tadpoles the granular glands are but metamorphosed mucous glands, and Wenig ('13) confirms his conclusion for the Anura, as he finds that in *Bufo vulgaris* the poison glands arise as modifications of the mucous glands.

b. Early stages in *Necturus*. In *Necturus*, according to Eycleshymer and Wilson ('10), the fundaments of the skin glands first make their appearance in the epidermis of the middorsal region of a 32-mm. embryo. In a 36-mm. embryo the glands are larger, are sunk below the level of the epidermis, and possess a lumen. In a 37-mm. embryo young glands are present on the sides and in the ventral region, both anteriorly and posteriorly. For larvae 43 mm. long, they describe the condition thus: "Mu-

cous glands larger, abundant, none on the ventral surface." The contradictory statements made by these writers regarding the distribution of the glands on the ventral surfaces of the 37- and 43-mm. embryos are probably due to the fact that the descriptions were based on animals which exhibited individual variation in the time of the development of the glands. From the meager account given it seems that the mucous glands must develop first and that they are limited in very young embryos to the dorsal region. I have not had an opportunity of studying embryonic material, but the adult animals and the 10-cm. larva upon which I worked furnished quite complete series of stages in the development of both types of glands. In the larva developing glands were numerous. In the adult they were found only occasionally. In all of the material examined no evidence was obtained to indicate that the granular glands were derived from those of the mucous type, but everything pointed to a separate origin and development for each kind of gland. The description of the development of glands in the larvae, with the exception of Phisalix ('00, '03), are all essentially alike. Maurer ('95), Ancel ('02), Nirenstein ('08), and Muhse ('09) have found that at frequent intervals a single cell in the lower cell layer of the primitive epidermis becomes differentiated. Rapid cell division follows and a solid mass of cells, the gland bud, is soon formed. This group of cells is at first contained in the epidermis and produces a bulging toward the dermis. Later the cell mass pushes down into the dermis, but retains its connection with the epidermis. Soon a lumen arises in the center of the mass, and at the point of connection with the epidermis the future duct is developed.

In the adult *Necturus* the youngest gland found was a bud consisting of about twelve cells which lay well within the dermis (fig. 17). The arrangement of the cells in the overlying epidermis was disturbed and open spaces were observed in the malpighian layer, indicating an active emigration of cells from that region. In this case, the developing gland entered the dermis along one of the perpendicular bundles of connective tissue. In figure 18 a somewhat later stage than that of figure

17 is represented. This consists of a solid mass of cells which is already assuming the form of the mature gland. Mitoses are frequent both in the gland bud and in the epidermis above. There is as yet nothing in the staining reactions or appearance of the cells to indicate whether this is the young stage of a granular or of a mucous gland.

c. Differentiation of the granular gland. Later developmental stages show the young gland considerably increased in size with a well-defined lumen around which the cells are now definitely arranged radially to its center. The increase in size is partially due to the formation of the lumen, but there is also an actual increase in the number of cells, due both to the division of the cells within the gland and to the immigration of cells from the epidermis. Maurer ('95), Ancel ('02), Nirenstein ('08), and others have described, in the larval development of the glands, an early differentiation of the cells of the bud into two layers—the outer to form the muscular wall, the inner to form the glandular epithelium. This condition is not realized in adult *Necturus*, the cells in early stages of gland formation being arranged in a single layer around the lumen and taking on a characteristic form and appearance which marks them as being potential granular cells (fig. 19). They are tall, columnar, and closely packed (figs. 23, 29). Their cytoplasm is very resistant to all stains, so that the epithelial layer at this stage always appears clear. Within the cells, and running parallel with their long axes, fine wavy lines can be distinguished. On the ends of the cells, peculiar club-like masses, composed of a clear non-staining substance unlike cytoplasm, extend into the lumen of the gland. These masses do not appear to be important in the life-history of the cell and generally disappear when secreting is begun. The nuclei are ellipsoidal and usually lie near the center of the cytoplasm, but they are sometimes nearer the bases of the cells.

Fine filaments have been described in developing poison cells by Schneider ('02), Nirenstein ('08), and O. Schultze ('11). These filaments show an affinity for basic dyes, especially iron haematoxylin, and are regarded by Schneider and Schultze as taking part in the formation of the secretion granules which, it

will be remembered, were found by many to blacken in iron haematoxylin. But Nirenstein denies that there is any relation between the secretion and the filaments. Most workers, however, are agreed that the granular secretion is differentiated in the protoplasm of the granular cells. Several investigators have maintained that the granular secretion is a direct product of the nucleus. According to Vigier ('00), the secretion granules in the poison glands of Triton arise within the nucleus and are later expelled into the cytoplasm. Phisalix ('00 a, '00 d) finds a condition very similar to this in the poison cells of the salamanders. Corti ('09) and Furlotti ('11) have confirmed Vigier's results, and Muhse ('09) appears to think that in the toad both cytoplasm and nuclei may elaborate secretion.

In *Necturus* the development of the secretion does not occur simultaneously in all the cells of the gland, but the cells develop individually or in groups of two or three (fig. 19) at a time. A cell which is coming to maturity and beginning to function enlarges tremendously and the clear filar cytoplasm becomes reticulated, the reticulum being first apparent in the region nearest the gland lumen. In these enlarged and reticulated cells fine granules, staining densely in eosin, make their appearance. The granules increase rapidly both in number and size and soon the entire plasma seems to be converted into an immense mass of secretion. The nuclei lose their ellipsoidal form and become more nearly round; they are gradually forced by the pressure of the secretion toward the base of the cells. The first cells to mature are usually in the bottom or fundus of the gland sac, those near the point of connection with the epidermis being the last to undergo the transformation. While these changes are going forward the cells rapidly increase in size and height until their central ends are in contact in the middle of the gland, thus doing away with all trace of a lumen (fig. 24).

Later, the cell limits entirely disappear and the cell contents flow together, giving rise to the syncytial condition already described in the mature gland. Figure 13 shows a developing gland in which the cell walls have been obliterated near the upper pole, but still persist near the bottom. As already stated,

in its early stages the secretion is readily fixed in all fluids, the fixed globules then appearing as large, more or less spherical granules. Sooner or later, however, it reaches such a condition that in Kleinenberg's, Flemming's, or other acid fixing solutions the large granules disintegrate. In some cases, the cell walls disappear before the change in the nature of the secretion occurs (fig. 19); in other cases, the secretion reaches the mature condition while the cell limits are still well defined (figs. 13, 24).

In figure 13 a large cluster of cells is seen at the upper pole of the gland in the region of the future duct. This condition suggests that cells are migrating downward from the epidermis faster than they can be accommodated by the developing gland, and reminds one of the epithelial plug described by Drasch ('94). In this gland an intercalary region is present. Also several flattened cells (fig. 13, *cl'*) are distributed over the periphery of the gland. They appear to be undifferentiated cells which have migrated into their present positions after the metamorphosis of the original glandular epithelium. In the peripheral regions of other glands, in which the epithelial cells have been transformed bodily into secretion, small gland cells, with distinct walls and filled with young secretion granules, are often observed. They probably represent later stages of the flattened cells shown in figure 13.

In young glands the intercalary cells appear to form a reserve from which granular cells are differentiated from time to time, and the supply of cells in this region is maintained either by mitosis or emigration of cells from the epidermis. Besides being continually reinforced in these later stages by epidermal cells which migrate downward by way of the intercalary region, the granular cells may themselves multiply by mitotic division (fig. 7). Binuclear or multinuclear conditions in the large granular cells have been described by several writers (Engelmann, '72; Drasch, '92, '94; Nicoglu, '93; Vollmer, '93; Talke, '00; Esterly, '04; Arnold, '05; Nirenstein, '08; Phisalix, '10). Schultz ('89) has described mitosis as occurring very frequently in the poison cells. Drasch ('92, '94), however, states that he has examined hundreds of preparations and has never found a single mitotic

figure. He concludes, therefore, that in late stages of gland development the increase in the number of nuclei takes place by direct division. Nicoglu ('93) and Nirenstein ('08) hold a similar view. According to Talke ('00), all increase in the number of cells, in the late as well as in the early stages, is a result of indirect division. In the glands of *Necturus* mitotic figures are found occasionally and in several cases granules of secretion could be distinguished scattered about the spindle figure, showing that, even after a cell has begun to elaborate secretion, the nuclei may divide by the indirect method. But division of the cell does not always immediately follow nuclear division, and two, sometimes four, nuclear masses are found within the limits of one cell. This condition is best demonstrated in tangential sections of the glands (fig. 10), where the nuclei may be seen in groups of two or four.

During the development of the secretion within the granular cells, the nuclei usually increase enormously in size, and those writers who have maintained a nuclear origin for the secretion regard this phenomenon as evidence in support of their view. Still, it is quite probable that with the increased activity within the cytoplasm there would be, correlated with it, an increased activity within the nucleus, which is commonly regarded as the dynamic center of the cell. The accelerated metabolism within the nucleus might well find expression in an increase in its size. However, many granular cells, containing immense quantities of secretion, have nuclei very little larger than those of ordinary epidermal cells from which they were derived (fig. 33). Also in mature glands, in which no new secretion is being formed and the cell limits have entirely disappeared, greatly enlarged nuclei are found (fig. 9). It would seem, therefore, that increase in the size of the nucleus is not always closely correlated with active production of secretion. In *Necturus* I have never found any acidophilous granules within the nuclei, and there is nothing to indicate that the secretion is a direct nuclear product.

In the glands of *Triton*, Klein ('79) has described giant nuclei from 126 to 129 μ long. Nicoglu ('93) also found these 'Riesenkerne' in the poison glands of several species of *Triton*. *Phisalix*

('00) describes the active nuclei as being from five to six times larger than those which are inactive, and practically all workers have reported a great increase in size in the nuclei of active cells.

The large nuclei found within the granular glands of *Necturus* have a normal structure and do not show any evidence of degeneration. The nuclear network is irregular but clearly defined and the nuclear membrane is distinct. The nuclei vary greatly in form. Some are ellipsoidal, while others are greatly flattened or elongated. A good idea of the various shapes assumed can best be given by a brief statement of the dimensions of some of the larger and more characteristic of these chromatic masses. The following measurements of length and breadth were made by means of the camera lucida and stage micrometer. Thickness was calculated from the number of sections in which a single nucleus appeared in a series of sections of known thickness. The largest nucleus was $260\ \mu$ long, but it was only $10\ \mu$ wide and was less than $6\ \mu$ thick (fig. 9). Another nucleus was $70\ \mu$ long, $44\ \mu$ wide, and $30\ \mu$ thick. Others were 100, 144, 164, and $172\ \mu$ long; 15, 15, 40, and $12\ \mu$ wide, and 8, 10, 10, and $8\ \mu$ thick, respectively. The outlines of all were quite regular and there was nothing to indicate that these giant forms had been developed from a coalescence of several nuclei. Occasionally large irregular chromatin masses were found where it was difficult to tell whether they represented single nuclei or were due to the close grouping of several. In one case the outline of six nuclei could be distinguished with certainty. Nicoglu describes similar 'Kernmasses' in *Triton cristatus* and states: "Diese Bilder können mit ziemlicher Sicherheit auf eine direkte Theilung des Zellkernes bezogen werden" (p. 448). It is probable that in *Necturus* they arise in the same way, but there was no evidence to indicate positively whether division took place by the direct or indirect method.

In the adult *Necturus* the ducts and muscular layers are the last parts of the gland to be developed, and they probably make their appearance at about the same time. Many large gland sacs are found with their epithelium completely transformed into secretion and the cell walls entirely gone, but possessing

neither duct nor a muscular layer (fig. 9). The muscle cells grow down from the intercalary cells and become differentiated into fibers about the outer surface of the gland sac. The duct is very simply formed and is equivalent to a large intercellular space. Apparently it does not always extend entirely to the surface of the epidermis, since in many cases the expulsion of the secretion results in an upheaval of the upper epidermal layers in the region immediately above the upper pole of the gland. According to Fano ('03), the granular glands in young *Amblystoma* function without a duct and the secretion is forced through the intercellular spaces of the epidermis to the outside.

The later appearance of muscles and ducts on the granular glands would seem to explain why, when a violent stimulus is applied to the surface of the animal, all the glands are not discharged at the same time. A well-developed muscular coat is always present on emptied glands. If muscles were developed early on all glands, all the secretion would probably be expelled, upon the reception of the proper stimulus, and the animal left without adequate protection.

d. Differentiation of the mucous gland. In very early stages of their development, as has been shown, it is not possible to distinguish between granular and mucous glands. Figure 25 shows the earliest recognizable stage of a mucous gland. A distinct lumen is present and the cells are arranged so as to form a low epithelium. The nuclei are spherical or ellipsoidal, show a distinct chromatin network and occupy the centers of the cells. The cytoplasm in some places appears homogeneous, in others, finely granular, and colors slightly in either Ehrlich's haematoxylin or thionin. A large cluster of cells, similar to those already described in the developing granular gland, is present in the region where the gland is connected with the epidermis.

Later stages (figs. 26, 27, 28) show the gland cells undergoing considerable changes in form and appearance. Stricker and Spina ('79) distinguish three stages in the development of the mucous glands, 'Ringstadien,' 'Mittelstadien,' and 'Pfropfstadien.' In the first stage, or 'Ringstadien,' the epithelial cells are low and surround an extensive lumen; in the last, or 'Pfropf-

stadium' the cells are very tall and practically fill the entire lumen. Between these two extremes lies the 'Mittelstadium.'

In *Necturus* similar conditions are found and the various forms of the epithelial cells in developing glands appear to be due to the elaboration of the secretion. Figure 26 shows a gland in which the formation of secretion is just begun. The cells are cubical, with central nuclei, and are arranged in a single layer about a wide lumen. Just external to the definitely arranged epithelium, flattened cells (*cl'*) occasionally are found. They are apparently of epithelial origin and probably represent cells which are migrating downward from the epidermis, later to take up positions in the mucous epithelium. As more secretion is formed the gland cells enlarge greatly and extend further into the lumen (fig. 27). The nuclei, which formerly lay near the center of the gland cells, are now found close to the gland wall, being forced outward and greatly distorted by the pressure of the secreted substance. In figure 28 a condition corresponding to the 'Pfpopfstadium' is shown. The lumen of the gland has completely disappeared. The cells are enormously enlarged and elongated, and their entire protoplasm seems in most places to be replaced by mucus. In the upper part of the gland are several cells which have ruptured at their outer ends and their slimy contents have streamed outward through the duct. In the mature glands, as already noted, the cell walls are usually gone, leaving the secretion free within the body of the gland. In a few cases, however, only the ends of the cells have disappeared and on the sides the cells are still definitely limited.

In mucous cells the process of secretion is commonly described as beginning with granule formation, the mucigen granules later becoming changed into clear vesicles of mucus. Many writers have described a distinct granular stage in the elaboration of the secretion in the mucous glands of the amphibian skin (Nicolu, '93; Nordenskiöld, '05; Arnold, '05; Nirenstein '08).

In *Necturus* the mucous secretion makes its first appearance in the cytoplasm near the free ends of the cells, but no distinct granular stage in its formation was observed. Many young cells, however, do appear finely granular, but this condition

resembles that often found in ordinary epidermal cells, and does not seem to be identical with the granular stage described by other writers. From the first the secretion presents the same appearance and gives the same staining reactions as do the contents of the mature mucous cells. In the early stages of its formation the mucin gathers near the free surface of the cells, and a mass of secretion is thus produced which is sharply marked off from the underlying cytoplasm. As more secretion is produced the nuclei are gradually forced to the bases of the cells, where they lie imbedded in a small amount of unchanged cytoplasm. The nuclei usually become much smaller and lose their spherical or ellipsoidal form. The chromatin network also vanishes and the nuclei appear as densely stained homogeneous masses. In late stages, while the cell walls are still intact, the entire mucous cell exhibits a vesicular structure, little or no cytoplasm can be distinguished, and the nucleus is found in one corner of the cell closely pressed against the gland wall (fig. 28). Later, the cell wall is ruptured and the secretion escapes.

4. *Physiology.* a. Of the granular gland. When *Necturus* is resting quietly in the aquarium, no secretion can be observed on the surface of the body, although the animal always feels slimy. A discharge of mucus can be obtained very readily by handling or any slight mechanical stimulation, but the granular secretion is expelled only in response to some violent stimulus, either mechanical, chemical, or electrical.

I have been unable to learn what constitutes a proper stimulus, under natural conditions, for the discharge of the granular glands. The results of artificial stimuli differ. Electricity applied to a limited area of the skin causes an expulsion of the secretion in that region alone. Severe mechanical stimuli, sufficient to cause writhing movements, usually induced a general discharge from the granular glands. Killing with chloroform in every instance produced a general expulsion of both mucus and granular secretion. Animals which were wrapped loosely in towels and placed in the water usually discharged large quantities of both kinds of secretion while they were endeavoring to free themselves from confinement.

Authors have quite generally agreed that the expulsion of the granular secretion is due to the contraction of the smooth muscles found about the gland sacs. Seeck ('91), however, believes that the expulsion of secretion is caused by the underlying skeletal muscles. Muhse ('09) has experimented with many toads and has found that there is no change of tension produced in the skin when the glands are discharging. She concludes, therefore, that the expulsion of secretion cannot be due to any action of the trunk muscles. Evidence in support of Muhse's contention is also furnished by conditions in *Necturus*, since, in the regions of the edges of the tail where granular glands are present in great numbers, no skeletal muscles occur, the glands lying in the loose connective tissue which fills the space between the integument on either side of the tail. Since in *Necturus* there are no smooth muscle fibers present in the dermis, other than those definitely arranged in the walls of the glands, we may conclude with a fair degree of certainty that the expulsion of the granular secretion is a direct result of the contraction of the muscles immediately about the gland. The appearance of the muscles of emptied glands and the manner of their innervation also support this view.

In *Necturus* the contraction of the muscles usually proceeds uniformly over the entire surface of the glands. Occasionally, however, deep furrows or constrictions in the gland bodies were noted. These are apparently due to differences in the rate of contraction of individual fibers. It will be recalled that the muscle fibers lie in a meridional direction on the gland sac and that the upper ends of the muscle cells on the distal portion of the gland are attached at the intercalary region located at the base of the duct. Contraction therefore operates in two ways. It draws open the lower end of the duct and at the same time reduces the volume of the gland sac. At first only the granular secretion is expelled, but, as contraction proceeds further, many epithelial nuclei are carried away from the muscular wall and are forced up the duct along with the secretion (fig. 20). In several glands where almost all the secretion had been expelled, solid cores of nuclei were found in the ducts, having been forced

into these positions by the pressure developed by the contracting muscles. In many cases, therefore, contraction appears to result in the almost complete obliteration of the gland lumen and the removal of a large proportion of the nuclei of the old secretory epithelium. However, there are always a few nuclei left within the gland sac and they are usually elongated on the side toward the muscular layer (fig. 20), indicating that originally they had been attached to this region and were pulled into the form they now assume as a result of the contraction of the muscular wall and its subsequent withdrawal due to the elasticity of the dermis.

Emptied glands, although their volume is greatly decreased, always retain their spherical or ovoid form. They never present the collapsed appearance described by Drasch ('94), Nirenstein ('08), Muhse ('09), Shipley and Wislocki ('15), and others. The muscle layer is always prominent and the intercalary region is drawn down and elongated. Often the epidermis above suffers considerable distortion and in many cases epidermal cells are torn away, due either to the force of the escaping secretion or to the incomplete development of the duct.

In undischarged glands the muscle fibers are very thin and greatly elongated, and can be distinguished with certainty only under the higher powers of the microscope. Their nuclei in perpendicular sections also appear long and thin. In the contracted condition, the fibers are easily seen, being greatly thickened and shortened. Their nuclei, too, undergo corresponding changes in form. In material killed and fixed immediately after the expulsion of secretion the contracted muscles show little fibrillar structure and stain intensely in eosin. In tissue prepared twenty-four to forty-eight hours after the emptying of the glands, myofibrillae are easily seen and the muscle fibers do not take the eosin so readily.

In the poison glands of *Salamandra maculosa*, Drasch ('94) described cross-striations on the contracted fibers and regarded them as being due to wrinkling of the *membrana propria* of the gland, and not to any change in the surface of the fibers themselves. Also in *Necturus* cross-striations were noted and are clearly shown in tangential sections of the gland in which the

muscles are seen in surface view. Figure 21 represents such a section. Near its center the entire muscle wall has been cut away, exposing an epithelial nucleus and some secretion. At the top the fibers have been sectioned somewhat obliquely to their long axes. Near the bottom the surface of the muscle fibers is barely exposed, and zigzag striae are seen extending across them. The markings remind one somewhat of the appearance presented by the outer edges of the myomeres of a fish when the muscle has been exposed by the removal of the skin. In more nearly median sections of the gland, where the fibers are seen in longitudinal section, the striations appear as ragged, tooth-like projections along the outer edges of the muscles. Further, around every contracted gland there are strained regions in the connective tissue caused by the enormous decrease in the volume of the gland. The striations therefore are probably due to some connection between the muscle fibers and the surrounding connective tissue, the resistance offered by the connective-tissue fibers as the muscles contract resulting in some way in the production of ridges on the outer surfaces of the latter. The zigzag or wavy course of these transverse markings is possibly caused by variations in the amount of contraction in individual fibers.

b. Of the mucous gland. As already noted, mucus appears on the surface of the animal very readily. Any slight mechanical stimulus will induce a discharge. The effects of handling and of killing in chloroform have already been mentioned. Ammonium hydroxide or other irritating reagents applied to a limited area, when the animal is out of water, cause a strong flow of secretion from the mucous glands in that region only. When the animal is returned to the water, secretion is still discharged, giving rise to cord-like masses of mucus which float up in the water. The quantity of mucus obtained by any vigorous chemical or mechanical stimulation is enormous, being many times greater in volume than the skin by which it is produced. The secretion apparently swells when it comes in contact with the water, and moisture seems to be necessary in order that mucus be discharged freely. If an animal be taken from the water and

left undisturbed no fresh mucus appears on the surface and the secretion already present dries, forming a thin transparent covering. (It, however, cannot afford much protection to the epidermis, since molting follows a prolonged drying in the air.) If a portion of the skin be wiped clear of mucus and a piece of dry filter-paper placed upon it, no secretion is discharged. If, however, the paper be kept moist, mucus appears beneath it in large quantities and soon saturates it.

The mechanics of the discharge of the secretion from the mucous glands is a disputed question. Nussbaum ('82) asserts that when the glands of the salamander are stimulated the inner ends of the secreting cells are discharged. According to Drasch ('89), secretion is a more or less continuous process and there is no accumulation of a great reserve to be expelled in an emergency as is the case with the larger, granular glands. He found that stimulation of the trigeminal nerve caused a contraction of the membrane about the gland, while stimulation of the sympathetic produced an increase in the volume of the mucous cells. Maurer ('95) thought the contraction of the perpendicular muscles of the dermis might have some effect on the glands, and Tonkoff ('00) suggested that the elastic fibers investing the glands might be concerned with the expulsion of secretion. Those investigators who have found a muscular layer about the mucous glands regard the contraction of the muscle fibers as the direct cause of the discharge of secretion.

In *Necturus* muscles are never found upon the mucous glands. Further, glands which are actively discharging seldom exhibit any evidences of contraction, but in some cases their ducts are very wide. No information whatever was obtained regarding the manner of the expulsion of the secretion, but, judging from the way it makes its appearance on the surface, it is forced out under some pressure, which must be due either to a tension outside of the gland or to changes of pressure within the gland itself.

C. Mixed glands

1. *In other Amphibia.* In the introduction to the discussion of the glands brief mention has already been made of the mixed glands and of the various views regarding their possible significance. Hoyer ('90) described a mixed condition for some of the poison glands of the salamander. Lying between the unstained 'Riesenzellen' of the granular gland, he found single cells or groups of cells which gave with thionin the specific color reaction for mucin. Mixed glands have been reported also by Heidenhain ('93 a) and Nicoglu ('93). According to these investigators, this 'Doppelbildung' is a result of replacement of the poison gland by one of the mucous type. Nicoglu suggests that the replacement occurs as an adaptation, because the animal, through some unusual change in environment, has come to need more mucus than could be secreted by the normal mucous glands. Talke ('00) confirms the conclusions of Heidenhain and Nicoglu, and describes a "Schleimsecernierendes Drüsen-säckchen in dem Balge der alten Giftdrüse." The last three writers referred to deny the possibility of a direct metamorphosis of the slime cells into poison cells, as suggested by Hoyer ('90).

Esterly ('04), in his work on the glands of *Plethodon*, describes small mucous sacs lying within the poison glands. He believes that, when the poison cells are exhausted, the mucous sac enlarges, replaces the poison gland, and very probably metamorphoses into a gland of the latter type. That is to say, the mucous cells may be directly differentiated into poison cells. Nordenskiöld ('05) has described a mixed condition in the toad "wo der Fundusteil der Drüse von Schleimzellen, die übrige Drüse von Giftzellen ausgekleidet ist" (p. 11). In another place, while discussing the relation of the two types of glands, he says: "Anderseits scheint jedoch in den meisten Fällen sehr früh eine bestimmte Sonderung der Funktion einzutreten, so dass sich die junge Drüse definitiv zur Schleimdrüse oder Giftdrüse ausbildet."

Nirenstein ('08), who worked on *Salamandra maculosa* and *Triton*, gives quite a different explanation of the small mucous sacs described within the lumina of the poison glands by Heidenhain ('93 a), Nicoglu ('93), Talke ('00), and Esterly ('04).

According to Nirenstein, the double structures arise in the course of the development of poison glands from mucous glands, and are not the result of any process of replacement of an exhausted epithelium by a new one.

Furlotti ('09, '11) also describes mixed glands in *Triton cristatus*. According to him, the gland epithelia are of two distinct types, and the mixed condition may be permanent. The granular portion of the gland is sometimes in communication with the duct and at other times the mucous secretion is discharged through the outlet. Weiss ('08, '15) states that in the frog the granular glands are directly differentiated from those of the mucous type and that in this process of differentiation mixed glands are temporarily formed. These double structures, however, are quite unlike those described in *Salamandra*, *Triton*, and *Plethodon*, since the mucous cells never appear as small sacs within the large granular glands. The mucous cells are found in the fundus of the gland and the granular cells lie near the base of the duct. This condition, according to Weiss, is due to the fact that the process of metamorphosis of mucous cells into granular cells begins at the duct, and the rapidity with which the metamorphosis is accomplished explains why mixed glands are so rarely found in preparations of the skin.

2. *In Necturus*. The mixed glands found in *Necturus* resemble those described by Nordenskiöld ('05) and Weiss ('08). The granular cells occur in the upper part of the gland, just below the intercalary cells, while the mucous epithelium usually occupies the deeper part of the gland alveolus (figs. 33, 37). All stages of the mixed condition are found, from one in which only a small quantity of granular secretion is present in the region of the gland neck to one in which the mucous epithelium and secretion have been almost completely displaced and the lumen is occupied chiefly by the granular secretion. In several cases fully developed granular glands were found with plugs of mucous secretion in their ducts. This probably represents the last stage of displacement of the mucous secretion by the granular secretion, and has been so interpreted by Weiss ('08). Calmels ('83) also speaks of a 'slime plug' in the ducts of some poison glands.

It has already been stated that Weiss ('08, '15) regards the mixed condition in frog tadpoles as arising from the metamorphosis of the mucous epithelium into a poison epithelium, the process beginning in the cells near the neck of the gland. Although the conditions found in *Necturus* resemble very closely those described by Weiss, still it seems impossible to adopt his interpretation of the origin of mixed glands. All the evidence obtained from a study of the glands of adult *Necturus* indicates strongly that we are not dealing with a metamorphosis of mucous cells into granular cells, but that we have granular cells being differentiated from the intercalary region, moving down all around the wall of the gland and gradually replacing the mucous epithelium. All the glands with a mixed epithelium possess a fully developed duct. The mucous cells which are being displaced are completely transformed into secretion, appearing as they do in mature functioning glands, with their walls entirely gone and their nuclei flattened and densely stained (fig. 37). On the other hand, the granular cells, especially those near the duct, usually appear young. The granular secretion is in the early stage of its elaboration and appears in all fixations as large discrete granules. The walls of the granular cells are distinct. Furthermore, on the side next the mucous secretion the granular cells are generally somewhat flattened and on this flattened surface lie the densely stained nuclei of the old mucous epithelium which is being displaced.

In figure 33 a later stage of displacement than that represented in figure 37 is shown. On the left side of the gland, immediately below the intercalary region, are two undifferentiated granular cells. They are clear and resistant to stain. Within their cytoplasm the fine wavy lines observed in the cells of developing granular glands can be distinguished. Below them is a large granular cell whose wall has disintegrated and allowed the granules to escape into the mucous secretion. On the right side of the gland is an immense granular cell, greatly distended with secretion, but with its cell wall still intact.

The conditions just described appear to furnish no evidence in support of the theory of the origin of granular cells from differentiated mucous cells. In all mixed glands found, the

mucous epithelium was completely metamorphosed into secretion and the cell walls were gone. It is difficult to think that poison cells, possessing definite walls and capable of producing immense quantities of secretion could arise from these completely differentiated and apparently almost exhausted mucous cells. It appears much more probable that in a mucous gland the intercalary region, itself of epidermal origin, could furnish cells which might develop under suitable conditions into granular cells, since the germinative layer of the normal epidermis produces gland buds which may become either granular or mucous glands. The displacement of the mucous nuclei from the gland wall and their subsequent position on the lumen-surface of the granular cells seems to furnish almost conclusive evidence that the granular cells are not developing from mucous cells, but are displacing them.

In one instance a peculiar mixed gland was found (fig. 36). Its appearance suggests that the mixed condition may in some cases become permanent. The gland possessed a well-formed duct and intercalary region. One half of the alveolus was occupied by the typical mucous epithelium and secretion. On the other half, a definite muscular layer apparently in the contracted state was present and, resting upon it, was a single layer of tall columnar cells which possessed all the characteristics of potential granular cells. The only possible interpretation of this structure seems to be that it is a permanent mixed gland with the granular and mucous epithelia existing side by side. Around the granular portion a muscular layer has developed, but, as would be expected, none has appeared around the mucous half of the gland. The presence of the clear epithelium resting upon a contracted muscular layer is difficult to explain. But in the light of conditions found in regenerating granular glands, to be described later, the most plausible explanation would be to regard the clear, columnar cells as a new epithelium which is replacing exhausted granular cells whose contents have been expelled by the contraction of the muscular wall.

D. Regeneration

1. *Of granular glands.* a. In other Amphibia. There is considerable diversity of opinion regarding the fate of the discharged glands, but it is almost uniformly admitted that the cells of the granular glands pass bodily into the secretion mass and that the expulsion of the secretion involves the death of the cells. However, Weiss ('99) and Nordenskiöld ('05) believe that, in the toad, the gland cells are not completely transformed into secretion and are, therefore, not destroyed, but that the protoplasm of the inner part of the cells furnishes new secretion and again fills the emptied sac. Calmels ('83) and Seeck ('91) describe replacing cells within the old glands, but, according to Muhse ('09) and others, they mistook cross-sections of smooth muscle fibers for young epithelial cells. Schultz ('89) states that the cells of the poison glands multiply by mitotic division and that only a few cells reach full development at the same time.

According to Engelmann ('72), Junius ('96), and Muhse ('09), the emptied glands degenerate and new glands are developed by the embryonic method. In many Amphibia, however, definite processes of replacement of the exhausted epithelium have been described. Production of secretion is, therefore, in some Amphibia maintained by a rehabilitation of the old gland, and not always, as Junius ('96) and Muhse ('09) claim, by the development of entirely new glands. In Triton, Nicoglu ('93) and Heidenhain ('93 a) find that inside the old poison gland there is present a second smaller gland possessing a lumen. This small sac lies between the musculature and epithelium of the large gland, and when the latter is emptied of its secretion, the bud enlarges and occupies the old lumen. The place of origin of the replacing gland is found by Heidenhain and Nicoglu to be in the small flattened cells immediately below the intercalary region. But Vollmer ('93), who also worked on Triton, concludes that the "Mutterboden der Drüsenknospe das Keimlager das Rete Malpighi ist."

Talke ('00) believes that in all Amphibia regeneration is a continuous process and takes place by mitotic division in the gland

epithelium. The frequency of mitosis in the old gland epithelium, coupled with entire absence of gland sacs from at least one-half of the large glands, has led him to conclude that regeneration by replacement was rather an exceptional occurrence.

In *Plethodon*, Esterly ('04) found that the method of renewal of the worn-out glands was the same as that described by Nicoglu ('93), Heidenhain ('93 a), and Vollmer ('93). He, however, obtained no evidence to show the source of the replacing glands, and, according to him, the bud is always mucous in character. Although Esterly has never observed transitional stages between the mucous bud and the poison gland, he believes the former replaces and assumes the function of the latter. For the frog, Arnold ('05) could not come to any definite conclusion regarding regeneration. In one case he found a small epithelial sac just within the neck of the gland and in several other places he noted small sacs with both muscle and secreting cells lying along the gland necks, but on account of their small size he was unable positively to demonstrate their relation to the old gland.

Nirenstein ('08) worked on *Salamandra maculosa* and *Triton*. He criticises the views of Heidenhain ('93 a), Nicoglu ('93), Vollmer ('93), and Esterly ('04) regarding the significance of the gland sacs. According to him, the gland sacs within the old glands are not concerned in regeneration, but represent stages in the development of the large gland. He claims further that sacs never appear of other than the mucous type and states that they represent portions of mucous epithelium which have not yet metamorphosed into poison cells.

Regarding the fate of the discharged granular glands, Nirenstein was in doubt. In many cases the old secreting cells gave unmistakable evidence of degeneration. The lumina of the glands were filled with numerous wandering cells, small mononuclear leucocytes, large polymorphonuclear leucocytes, large eosinophilous leucocytes, and pigmented wandering cells. Besides degenerative changes, regenerative activities are also observed, the latter occurring in the muscle cells of the glands. Rapid mitotic divisions took place in this layer and locally within many glands several layers of cells were produced. Fur-

ther than this Nirenstein was unable to trace the process, but four months after the discharge of the glands no trace of the empty sacs could be found. Further, he found that other glands, discharged at the same time, did not show any degenerative changes and "das ganze Aussehen der Drüse spricht für die Lebens- und Entwicklungsfähigkeit des Organs" (p. 105).

In *Bufo agua*, according to Bristol and Bartelmez ('08), "When the poison is discharged, the remains of the glands are resorbed, and at the same time one of the five or six undeveloped glands, grouped about the mouth of the functioning gland, grows down alongside the remains of the discharged gland pushing it aside to occupy its former place." Shipley and Wislocki ('15), who worked on the same animal, confirm the statements.

b. In *Necturus*. In *Necturus* the granular cells are transformed bodily into the secretion and the cell walls disintegrate, converting the gland sac into a reservoir for secretion. During the expulsion of the secretion the nuclei of the secreting cells are pulled away from the muscular layer and many are forced through the duct to the exterior (fig. 20). In most cases the whole appearance of the emptied glands would lead to the conclusion that their time of functional activity is at an end. However, in a few cases I found discharged granular glands that showed unmistakable signs of a renewal of secretory activities. Within them were small masses of newly elaborated secretion, easily identified by its appearance when fixed, but there was no evidence to indicate the source of the secretion. It may have been produced by some cells which had not been completely converted into secretion at the time the gland was emptied or, as some authors have suggested, it may have been the direct product of the naked nuclei, although there was absolutely no evidence that the nuclei were secreting.

In many glands, on the other hand, no signs of renewed activity by the remnants of the old epithelium were observed, and the nuclei of the old secreting cells were in a state of disintegration. Furthermore, many leucocytes of both the mononuclear and polymorphonuclear types, as well as pigmented wandering cells,

were found within the gland lumina, and it was evident that the remains of the secreting cells were being removed (fig. 22). Meanwhile, in these discharged glands considerable activity is noted at the intercalary region and, as the old epithelium is removed, there is developed from this position a new secretory epithelium. In figure 22 an early stage in this replacement of the old epithelium by a new one is shown. From all around the intercalary region cells move down along the old gland wall, forming a continuous layer. Apparently the intercalary region is unable to furnish enough cells for the complete repair of the gland, and after this supply has been exhausted, cells migrate downward directly from the epidermis not only from the germinative layer, but also from the transitional layers around the duct. Evidence for this conclusion is supplied from conditions like that shown in figure 23. In this gland the intercalary region has disappeared and in the duct the epidermal cells present an amoeboid appearance. Further, in other glands in which a new epithelium has been completely developed, the duct is very large, indicating that some of the cells now found in the body of the gland may have migrated down from its sides. Mitosis may also play some part in increasing the number of cells (fig. 29).

The epithelial cells derived from the intercalary region and epidermis gradually move downward and are arranged eventually to form a single layer over the entire muscular wall which is seemingly preserved intact. The new cells are soon definitely orientated radially to the center of the gland lumen, and assume the form and appearance already described for potential granular cells. They are closely packed, tall, and columnar. Their cytoplasm is clear and marked with fine lines, and on the ends of the cells clear, club-like masses project into the lumen (fig. 23). Later stages (fig. 29) show the gland cells becoming reticular and beginning to elaborate secretion. In fact, the replacing epithelium appears to follow exactly the developmental stages already outlined for the gland bud which arose as an invagination from the germinative layer of the epidermis.

In the discussion of mixed glands reference was made to what was apparently a permanent mixed condition (fig. 36). It will

be remembered that on one side of the gland sac a new undifferentiated granular epithelium was found resting on a completely developed muscular layer. This, as already suggested, is best explained by assuming that the granular half of the gland had functioned, and that, the old epithelium having been destroyed, a new replacing epithelium had moved down. The correlation between the development of a muscular wall and the presence of granular cells, contrasted with the lack of muscle fibers on the mucous portion of the gland, strengthens the conclusion that mucous glands never possess a muscular layer.

At no time in their development do the replacing cells give any evidence of being of mucous character and they never are arranged to form a small tubular sac within an unemptied granular gland, as in Triton. The regenerative process is never initiated till the fully elaborated granular secretion has been expelled and the remnants of the old gland cells are being removed. Regeneration of the granular glands in *Necturus*, therefore, is unlike that described for any other Amphibian, and also furnishes additional proof that the granular and mucous types of glands are entirely separate and distinct from each other in development as well as in histological structure.

2. *Of mucous glands.* a. In other Amphibia. Few authors have given any attention to the regeneration of the mucous glands. They have generally regarded them as producing secretion more or less continuously and have usually dismissed the subject by assuming that the individual cells function for a considerable time and, eventually becoming exhausted, are replaced, probably through mitosis. Talke ('00), who has investigated rather carefully the processes of regeneration in the glands of many Amphibia, concludes that the supply of mucous cells is in all cases maintained by indirect division. Esterly ('04, p. 243), in referring to the mucous glands, says: "It seems correct to say that the processes there are like those in milk glands where parts of the cells bodies are thrown off as secretion, while the remaining portions in time repeat the same processes of secretion."

Arnold ('05), in his discussion of regeneration of the mucous glands, does not come to any definite conclusions, but merely

presents several possibilities for the reader's consideration. 1) The cells may not be destroyed in secretion, but rejuvenate themselves from the non-metamorphosed part of the cytoplasm. In many cases he has found remnants of cytoplasm in the region of the nuclei. 2) Some cells may be destroyed in secretion and their loss offset by the differentiation of cells from the intercalary region. Mitotic figures were seldom observed and there was no evidence of amitosis. He suggests that the time of the year may have some influence on the regenerative processes in the glands. 3) "Die Drüse wird durch zapfenförmiges Einwachsen vom Oberflächenepithel aus total neugebildet." By this he apparently means that within an exhausted gland sac new epithelial cells may be developed by a downgrowth from the epidermis.

b. In *Necturus*. The mucous glands of *Necturus* never exhibit any evidences of the degeneration of their epithelium such as were found in the granular glands. The cells probably function for a considerable period before they are destroyed. New cells may be differentiated from the intercalary cells, since mitotic figures are occasionally found in that region. The lumina of the glands are seldom free from mucus. This indicates that secretion is produced more or less continuously. In a few cases, however, mucous glands are found in which the lumen is almost free from secretion and the secreting epithelium appears to be in a somewhat exhausted condition (fig. 32). In these the ends of the cells next the lumen lack a limiting membrane, but on the sides of the cells the walls are distinct. The cytoplasm containing the flattened, densely stained nuclei is confined to a narrow zone next the gland wall. The remaining portions of the glands appear empty, but the intercalary cells are highly developed, suggesting some activity in this region. It is difficult to explain satisfactorily the condition represented in figure 32, since cell walls are seldom found to persist in functioning glands. In this case the gland cells have apparently elaborated secretion and discharged it, leaving the cell walls intact except at the ends next the lumen. The general appearance of the gland, moreover, would lead one to believe that its time of functional activity is not at an end and that it is still capable of producing secretion.

INNERVATION

In the study of the nerves of the skin of *Necturus* the best results were obtained from the pyridin-silver nitrate-pyrogalllic acid method of Ranson. This method does not affect the sheaths of the nerves, but when successful leaves the axis cylinders black.

In the subcutaneous connective tissue the nerves form a wide-meshed plexus. From this small bundles pass outward, some going to the glands, others continuing to the epidermis. No evidence was found of the presence of a stratum of nerve cells, such as Herrick and Coghill ('98) and Coghill ('99) have shown.

Esterly ('04) found in *Plethodon* nerve fibers running for long distances beneath the dermis, and in many places they could even be seen to turn toward the epidermis, but all trace of them was lost as soon as they entered the dermis.

In *Necturus* small bundles can be readily followed through the dermis, but they can seldom be traced directly into the epidermis. This is due, in many cases, to the fibers' turning at right angles at the outer surface of the dermis and running for some distance parallel to the surface of the body before turning outward into the epidermis. Within the epidermis nothing but free intercellular fibers were found. These were seldom branched and were never found to terminate in small knobs or plates such as have been described for the epidermal fibers of many Amphibia.

Owing to the fineness of the fibers, the distribution of the nerves to the glands is not very apparent. This is especially true in sections passing through the median portions of the gland. However, in sections perpendicular to the surface of the body cutting the glands tangentially and exposing the surface of their walls, the nervous elements are easily distinguished. In no case was there found a network of fine fibers closely investing the gland, but at frequent intervals small bundles, usually consisting of from four to six nerve fibers, were seen leaving the nerve plexus below and coming toward the glands. In those of the mucous type these nerve fibers spread out over the outer surfaces of the gland cells and branch only occasionally. No evidence of intra- or intercellular endings was obtained.

On the granular glands (fig. 31), on the other hand, some fibers go directly to the muscle cells, ending in small round plates or bulb-like expansions (Huber and DeWitt, '98; Herrick and Coghill, '98; Coghill, '99; Esterly, '04), while other smaller fibers branch frequently over the surface of the muscular layer and then send small branching twigs between the contractile cells into the gland (fig. 30). These intraglandular endings probably represent interepithelial free nerve endings which supplied the granular epithelium when it existed as distinct cells before a muscular layer had developed. No perinuclear baskets of nerve fibers, such as Esterly ('04) has described for the poison glands of *Plethodon*, were observed.

SUMMARY

Epidermis

1. In the epidermis the cells of the transitional layers, as well as those of the so-called germinative layer, multiply by mitosis.

2. No basement membrane is present, but from their lower ends the cells of the germinative layer send out fine processes, which blend with underlying connective tissue of the dermis.

3. Except upon the hands and feet the free surface of the cells of the epidermis is provided with a dense top-plate, the so-called cuticular margin.

4. The cuticular margin is perpendicularly striated. The striations are interpreted as the walls of the closely packed tubular alveoli which make up this region.

5. The cuticular margin is, at least in part, a secretion product.

6. In molting, only the cells of the cuticular layer are cast.

7. A true horny layer is found only on the hands and feet.

8. Unicellular glands, commonly spoken of as club cells, are very abundant in the epidermis. Their exact function is unknown. There is some evidence to indicate that they discharge directly on the surface of the epidermis.

9. Typical goblet cells are found around the lips, in the epithelium of the mouth, and within the gular fold.

Epidermal pigmentation

1. Pigment may be present in ordinary epidermal cells. Collections of black granules are often found around that pole of the nucleus which is directed toward the outer surface.

2. The special pigment-bearing cells of the epidermis are of two apparently distinct types, 1) pyramidal and, 2) highly branched. There is some evidence that the two types may be but contraction and expansion phases of one kind of cell.

3. The epidermal chromatophores are not derived from those of the dermis.

4. Pyramidal cells often contain fragments of extraneous chromatin and cytoplasm, suggesting that they are of leucocytic origin.

5. In regeneration and transplantation experiments the epidermal chromatophores behave like epidermal cells. Specialized pigment cells may perhaps be produced by a metamorphosis of ordinary epidermal cells.

Dermis

1. The dermis is arranged in three layers, an outer compact layer, an intermediate spongy layer, and an inner compact layer.

2. Elastic fibers, both parallel and perpendicular to the surface, occur in the inner compact layer of the dermis. In the intermediate spongy region the elastic fibers are irregularly arranged, following in general the course of the connective-tissue bundles. None are found within the thin outer layer.

3. Melanophores and xanthophores are present in the dermis. The melanophores are usually arranged in two layers, one layer being immediately beneath the outer compact stratum, the other beneath the inner compact stratum. The xanthophores are commonly found with the melanophores of the outer layer.

Changes in coloration

1. Changes in the coloration of *Necturus* are produced by the contraction and expansion of the dermal melanophores.

2. In bright light the melanophores are always completely expanded.

3. In darkness at low temperatures the melanophores are contracted.

4. In bright light the color of the background has no appreciable effect. In twilight the melanophores are usually partially contracted, and on a white background the amount of contraction is increased.

5. In bright light changes in temperature produce no noticeable effects. In darkness, temperatures of 25°C. and over inhibit the contracting influence of darkness and the melanophores remain expanded.

6. Intracoelomic injections of adrenalin cause a complete contraction of the melanophores.

7. The melanophores appear to be of fixed form, within which the pigment granules migrate in 'contraction' and 'expansion.'

Glands

1. The skin glands of *Necturus* are of two kinds, granular and mucous. The mature glands are distinguished by the character and staining reactions of their secretion and by other histological features.

2. The bodies of the granular glands are invested by a muscular wall. Muscles are never found about the mucous glands.

3. In all mature glands the cytoplasm of the secreting epithelium is greatly reduced and the cell walls usually disappear. The nuclei of the granular cells are commonly spherical or ellipsoidal and stain normally. The nuclei of the mucous cells are smaller, irregularly shaped, and stain intensely.

4. The secretion of the granular glands stains readily in all plasma stains and is unaffected by basic stains.

5. The mucous secretion is never found in the form of granules. It is untouched by plasma stains, but is colored by thionin, resorcin-fuchsin, Delafield's or Ehrlich's haematoxylin.

6. Both types of gland develop by downgrowth from the germinative layer of the epidermis and are indistinguishable in very early stages.

7. With the appearance of a lumen the gland cells assume a definite form and appearance, which marks them as potential

granular or mucous cells. The granular cells never pass through a mucous stage before becoming completely differentiated.

8. The granular secretion is elaborated within the cytoplasm, which is eventually completely transformed into secretion. The lumen is obliterated by the enormous increase in the size of the cells, whose walls later disintegrate, converting the body of the gland into a reservoir of secretion.

9. The mucous secretion appears first as small vesicles in the cytoplasm. With the production of secretion the cells enlarge greatly and extend far into the lumen, and in many cases practically fill it. The cell walls usually disappear in late stages.

10. Giant nuclei are commonly found in developing granular glands, the largest observed being 260 μ long, 10 μ wide, and 6 μ thick.

11. Muscles of the granular glands are not developed till late. They are differentiated from the intercalary cells and are consequently of ectodermal origin.

12. Mucus appears on the surface of the body in response to very slight stimuli. More violent stimuli induce proportionately larger discharges.

13. Granular secretion is discharged only in response to very vigorous mechanical, chemical, or electrical stimulation.

14. The mechanism of the discharge of mucus is unknown. Granular secretion is expelled from the gland by the contraction of investing musculature.

15. Mixed glands, partly granular and partly mucous, are occasionally found. They represent stages in the displacement of the mucous epithelium by a new granular epithelium, which is differentiated from the intercalary cells.

16. In the elaboration and expulsion of the secretion the granular epithelium is usually destroyed.

17. Regeneration is accomplished by the downgrowth of a new epithelium which is derived from the intercalary region and the epidermis above. Leucocytes remove the remains of the old epithelium. The muscle layer is preserved intact.

18. The manner of renewal of the mucous glands is not very apparent. The individual cells may function several times.

New cells may be differentiated from the intercalary region. Mitosis is occasionally observed in the secreting epithelium.

Innervation

1. The epidermis is supplied with free nerve endings which are seldom branched.
2. Both the musculature and the epithelium of the granular glands have a direct nerve supply.
3. Branching fibers occur over the outer surface of the secreting epithelium of the mucous glands. There are no intra- or intercellular endings.

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DESCRIPTION OF PLATES

ABBREVIATIONS

<i>alv.</i> , alveoli	<i>leu'cyt.</i> , leucocyte
<i>chr'ph.</i> , epidermal chromatophore	<i>lu.gl.</i> , lumen of gland
<i>chr'ph'</i> , dermal chromatophore	<i>marg.cta.</i> , cuticular margin
<i>cl'</i> , flattened cells	<i>mit.</i> , mitosis
<i>cl.clav.</i> , club cell	<i>mu.</i> , skeletal muscle
<i>cl.grn.</i> , granular cell	<i>nl.</i> , nucleus
<i>cl.i'cal.</i> , intercalary cells	<i>nl.grn.</i> , nucleus of granular cell
<i>cl.muc.</i> , mucous cell	<i>nl.mu.</i> , nucleus of muscle fiber
<i>cl.pig.</i> , pigment cell	<i>nl.muc.</i> , nucleus of mucous cell
<i>cl.poc.</i> , goblet cell	<i>par.cl.</i> , cell wall
<i>cl.tis.co'nt.</i> , connective-tissue cell	<i>pig.</i> , pigment
<i>drm'</i> , outer compact layer of dermis	<i>pns.i'cl.</i> , intercellular bridges
<i>drm''</i> , intermediate spongy layer of dermis	<i>prc.cl.</i> , cell processes
<i>drm'''</i> , inner compact layer of dermis	<i>sec.grn.</i> , granular secretion
<i>dt.</i> , duct	<i>sec.muc.</i> , mucous secretion
<i>e'drm.</i> , epidermis	<i>spa.lym.</i> , lymph spaces
<i>fbr.ela.</i> , elastic fibers	<i>str.</i> , striae of cuticular margin
<i>fbr.mu.</i> , muscle fibers (smooth)	<i>tab.sec.</i> , secretion plates of cuticular layer
<i>fbr.n.</i> , nerve fibers	<i>tis.co'nt.</i> , connective-tissue bundle
<i>gl.grn.</i> , granular gland	<i>tis.co'nt'.</i> , vertical connective tissue bundle
<i>gl.muc.</i> , mucous gland	<i>tis.co'nt''</i> , subcutaneous connective tissue
<i>gm.gl.</i> , gland bud	<i>trm.n.</i> , nerve ending
<i>grn.</i> , granules in cuticular cells	<i>va.sng.</i> , blood-vessel
<i>la.crn.</i> , horny layer	<i>vac.</i> , vacuole
<i>la.cta.</i> , cuticular layer	
<i>la.g.</i> , germinative layer	
<i>la.t'i.</i> , transitional layers	

PLATE 1

EXPLANATION OF FIGURES

1 Section of epidermis perpendicular to the surface of the body from the region of the dorsal edge of the tail, showing the arrangement of the cells in layers, the cuticular margin, mitosis, and the distribution of pigment in ordinary epidermal cells, in highly branched chromatophores and in pyramidal cells. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 267$.

2 Epidermal chromatophore containing a densely stained protoplasmic mass. Zenker; Ehrlich's haematoxylin and eosin. $\times 340$.

3 Portion of epidermis from the edge of the tail with a pyramidal pigment cell discharging on the surface. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

4 Pyramidal pigment cell, with cytoplasmic and nuclear inclusions, in a vacuole of the epidermis. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

5 Section of epidermis perpendicular to the surface of the body from the side of the tail, showing a leucocyte lying within a club cell. Gilson; Ehrlich's haematoxylin and eosin. $\times 340$.

6 Section of integument perpendicular to the surface of the ventral region of the body showing epidermis, dermis, subcutaneous connective tissue, skeletal muscles, pigment, elastic fibers (in black), mucous glands, and a mature granular gland in median longitudinal section. Somewhat diagrammatic. Kleinenberg; Weigert's resorcin-fuchsin and van Gieson's picro-fuchsin. $\times 73$.

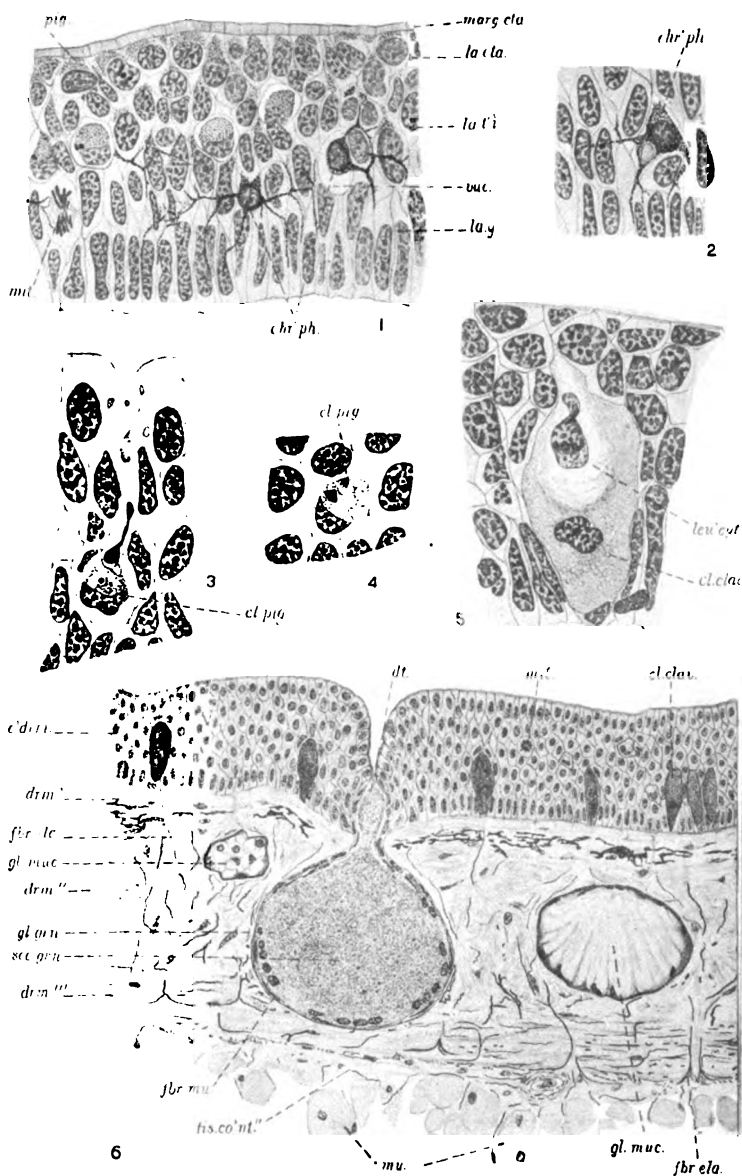


PLATE 2

EXPLANATION OF FIGURES

7 Section of a granular gland, showing mitosis in secreting cells at the bottom of the alveolus. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 178$.

8 Surface view of the cuticular margin, showing the polygonal areas (ends of alveoli). Drawn from an unstained preparation of the cuticular layer. $\times 733$.

9 Tangential section of a granular gland in which the muscular layer is not yet developed, showing a giant nucleus. $\times 178$.

10 Section similar to that represented in figure 9, showing a multinuclear condition in some granular cells and the grouping of nuclei in twos and fours in other parts where the cell walls have disintegrated. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 178$.

11 Epidermis from the toe, showing the horny layer and the cell processes of the germinative layer which extend into the dermis. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 178$.

12 Section of integument, perpendicular to the surface, from the dorsal side of the head, showing epidermis, three layers of dermis, subcutaneous connective tissue, pigment and a mature mucous gland in median longitudinal section. Somewhat diagrammatic. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 73$.

13 Median longitudinal section of a developing granular gland, showing a cluster of cells in the region of the connection with the epidermis. Intercalary cells present. Cell walls gone in the upper portion of the gland sac. At the bottom are two flattened cells just external to the definitely arranged epithelium. Kleinenberg; van Gieson. $\times 178$.

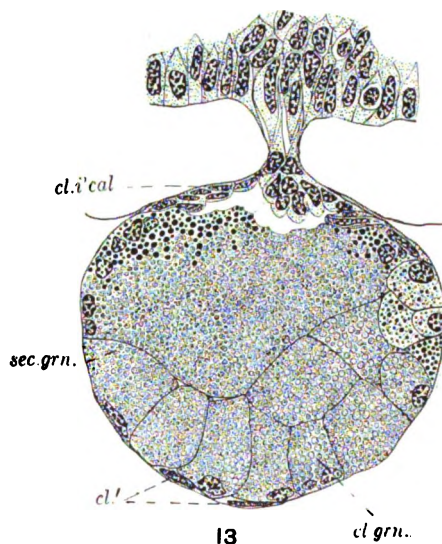
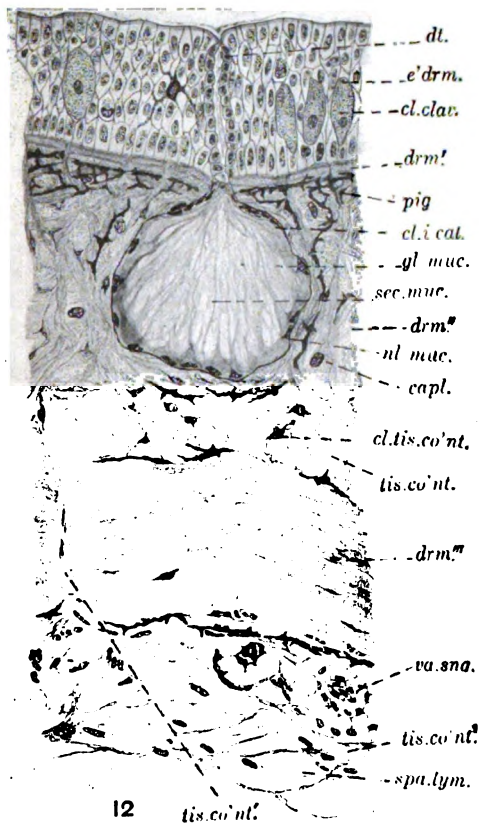
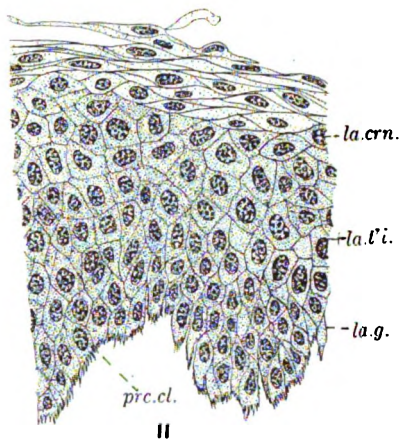
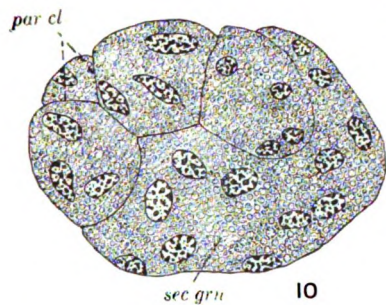
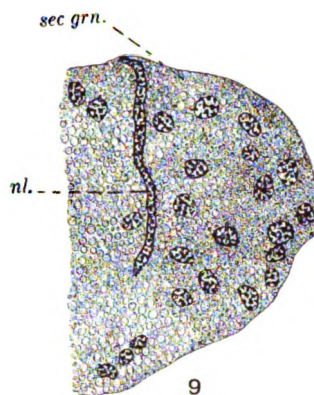
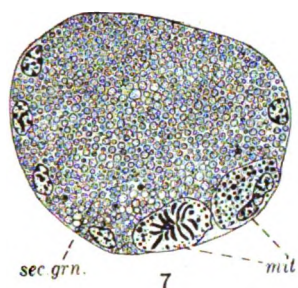


PLATE 3

EXPLANATION OF FIGURES

14 Club cells in the epidermis from the side of the tail. Zenker; Ehrlich's haematoxylin and eosin. $\times 178$.

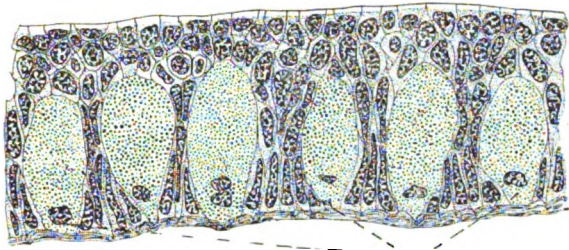
15 Three cells of the cuticular layer together with two replacing cells seen in longitudinal section, showing perpendicular striae, secretion plates (in surface view and in section), and secretion granules. Kleinenberg; Weigert's resorcin-fuchsin. $\times 733$.

16 The epidermis of the gular fold in perpendicular section, showing the reduction in the number of cell layers and a well-developed goblet cell. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 405$.

17 Early stage of a developing gland from the dorsal side of the tail. The epidermal cells are seen entering the dermis along a perpendicular bundle of connective tissue. No intermediate spongy layer is present. The two compact dermal layers lie close together. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

18 Later stage in the development of a gland in the region of the tail. The bud is solid. Mitoses in gland and in epidermis. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

19 Median longitudinal section of a developing granular gland from the region of the dorsal edge of the tail. A distinct lumen is present. The epithelium is definitely differentiated and considerable secretion has been produced. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$. (Successively later stages appear in fig. 24, pl. 4, and fig. 13, pl. 2.)



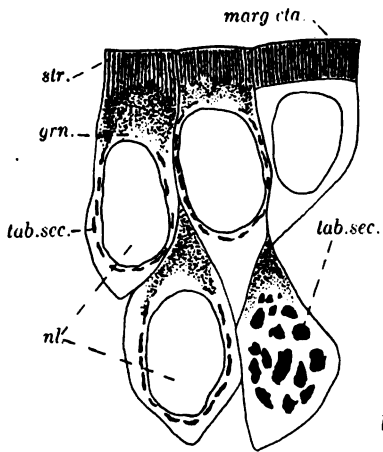
14

cl. clar



18

gm gl. mit.



15

marg. cla.

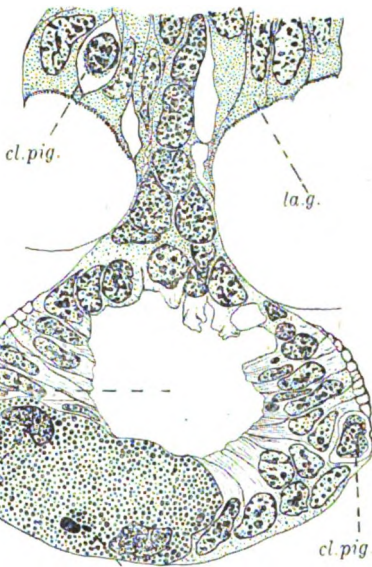
str.

grn.

tab. sec.

tab. sec.

nl.



19

cl. pig.

la. g.

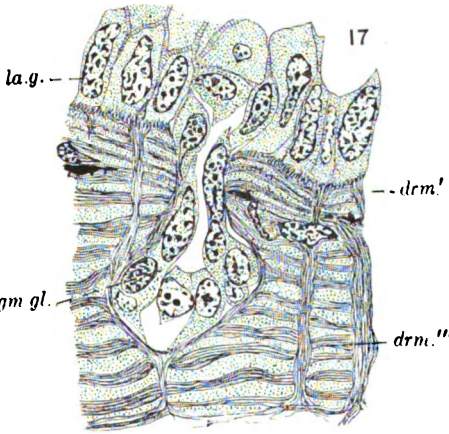
cl. grn.

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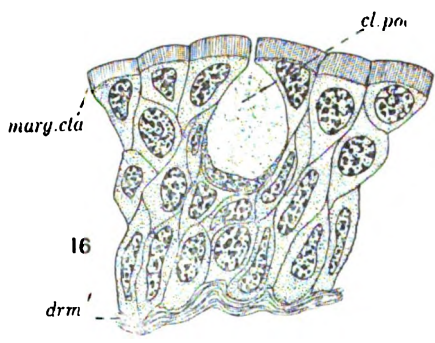
cl pig

sec grn.



drm'

drm'''



16

cl. pos

marg. cla.

drm'

PLATE 4

EXPLANATION OF FIGURES

20 Median longitudinal section of a discharging granular gland from the dorsal region of the tail. Granular secretion and many nuclei expelled, muscle layer prominent, epidermis deformed by the contraction of the gland wall. Zenker; Ehrlich's haematoxylin and van Gieson. $\times 178$.

21 Tangential section of an emptied granular gland, showing the transverse markings on the outer surface of the contracted muscle fibers. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

22 An early stage in the regeneration of an emptied granular gland. The section is not quite perpendicular, but passes somewhat obliquely through the lower end of the duct. Muscle layer intact, numerous leucocytes and pigmented wandering cells in the lumen, new epithelium developing from the intercalary region. Gilson; Ehrlich's haematoxylin and eosin. $\times 267$.

23 Median longitudinal section of a regenerating granular gland. Somewhat later stage than figure 22. Old muscle layer prominent; epithelium definitely arranged and typically granular. Epidermal cells along the duct amoeboid. Kleinenberg; Ehrlich's haematoxylin and van Gieson. $\times 267$.

24 Later stage of a developing granular gland, from the dorsal surface of the body, seen in median longitudinal section. Cells large and filled with secretion. Cell limits distinct, but the lumen obliterated. Within one cell the secreting granules are disintegrated. Neither muscles, intercalary region nor duct developed. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 178$.

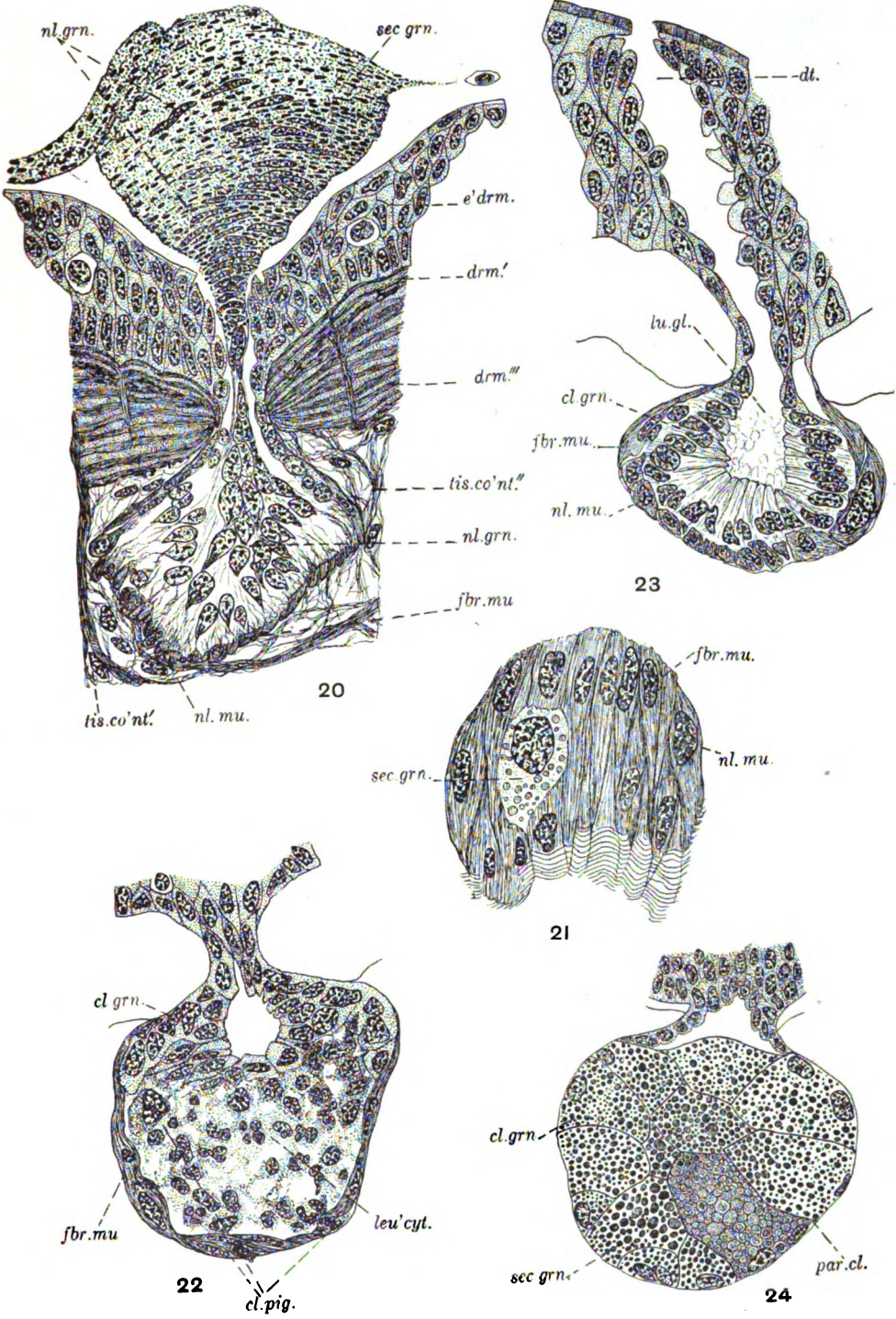


PLATE 5

EXPLANATION OF FIGURES

25 Early stage in the development of a mucous gland seen in median longitudinal section. Lumen present, cluster of cells at the region of connection with the epidermis. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 340$.

26 Later stage in the development of a mucous gland. Cubical cells arranged in a single layer about the lumen. Two flattened cells are shown just external to the definitely arranged epithelium. Duct partially formed. Kleinenberg; thionin and eosin. $\times 178$.

27 Mucous gland in which the cells are actively secreting. Cells are seen migrating downward from the epidermis. No duct yet developed. $2\frac{1}{2}$ per cent formaldehyde; thionin and eosin. $\times 340$.

28 Median longitudinal section of a mucous gland from the ventral surface of the body. Duct fully developed; cells very large and filled with secretion; nuclei small, irregular and densely stained. Near the duct some of the gland cells are ruptured. $2\frac{1}{2}$ per cent formaldehyde; thionin. $\times 178$.

29 Later stage in the regeneration of a granular gland. Note the wide duct, mitosis, absence of a definite intercalary region, and the pigmented wandering cell between cells of the gland epithelium. Secreting begun by the new epithelium. Clear club-like masses on the ends of the younger cells extend into the lumen. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 267$.

30 Portion of wall from a longitudinal section of a granular gland, showing the entry of free nerve fibers between the muscle cells. Ranson's method. $\times 405$.

31 Tangential section of the wall of a granular gland, showing nerve endings on muscles. Ranson's pyridin-silver nitrate-pyrogallie acid. $\times 405$.

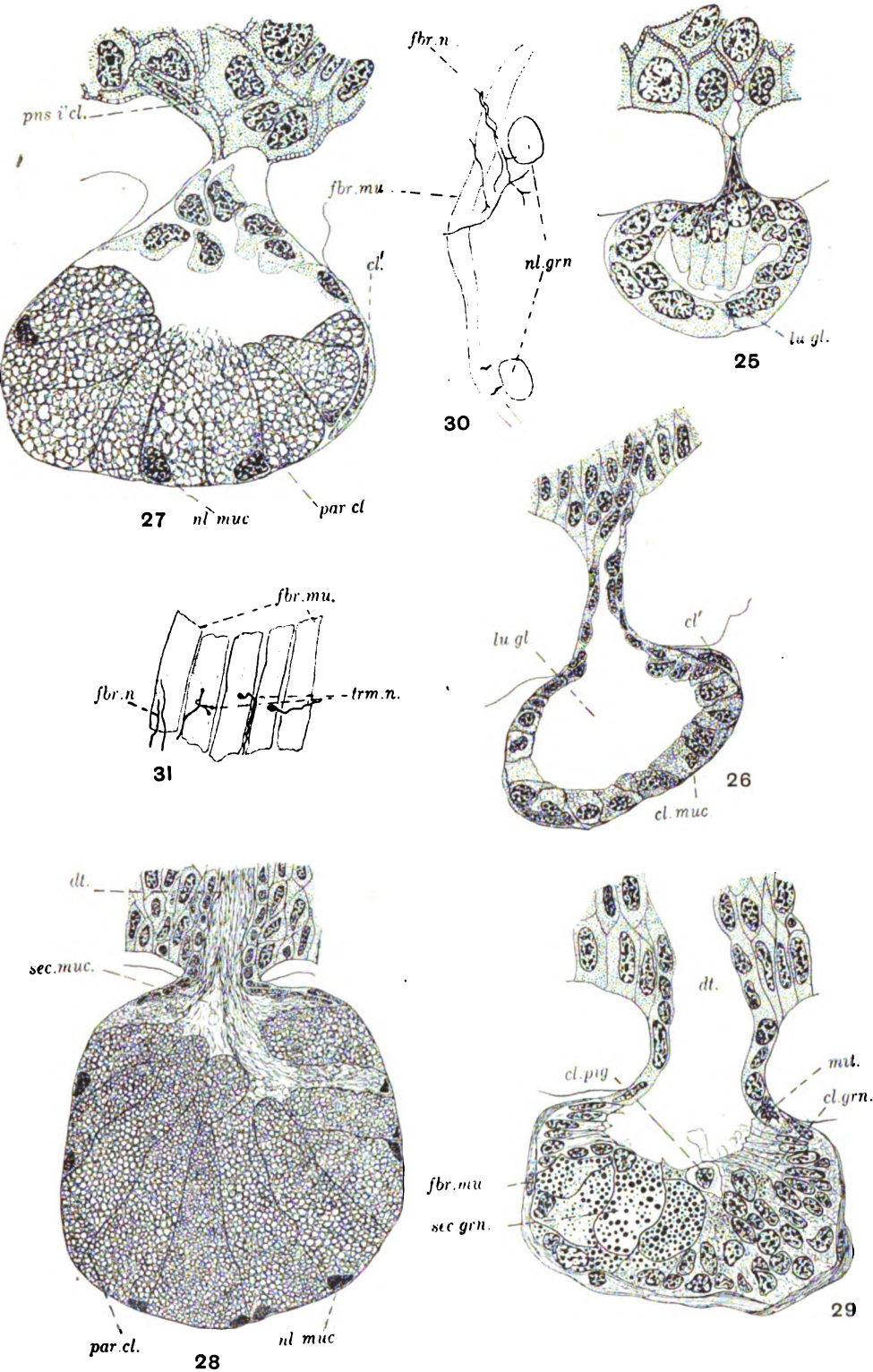


PLATE 6

EXPLANATION OF FIGURES

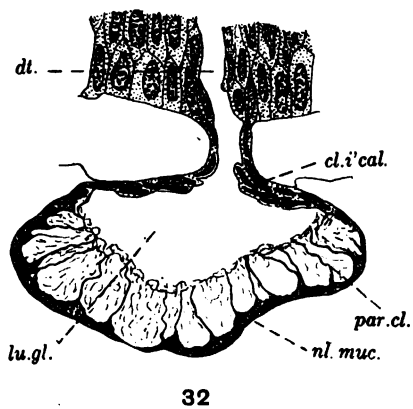
32 Mucous gland empty of secretion, shown in median section. Cell walls present except on the ends next the lumen. Nuclei basal and flattened. Duct completely developed and the intercalary region prominent. Zenker; Mallory's connective-tissue stain. $\times 178$.

33 Mixed gland, showing the granular cells greatly enlarged and distended with secretion. Kleinenberg; thionin and eosin. $\times 178$.

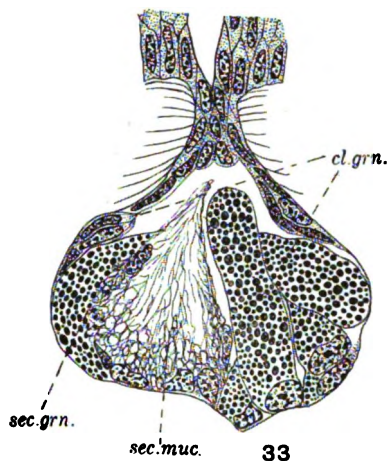
34 and 35 Surface views of dermal (fig. 34) and epidermal (fig. 35) melanophores drawn at the same magnification from unstained whole mounts of integument. $\times 178$.

36 Mixed gland; condition probably permanent. One half (right) consists of mucous epithelium and secretion. In the other half there is present a contracted muscular layer and young granular cells. Duct well developed, the nuclei of the cells lining it densely stained. Mucus is being discharged. Kleinenberg; thionin and eosin. $\times 267$.

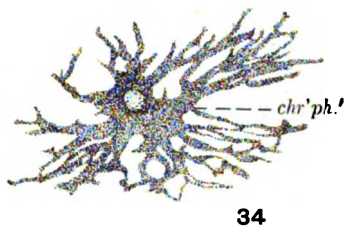
37 Mixed gland in which the mucous epithelium and secretion have been displaced by a granular epithelium developed from the intercalary cells. Mucous epithelium in the lower portion of alveolus; granular cells near the intercalary region. Kleinenberg; Ehrlich's haematoxylin and eosin. $\times 178$.



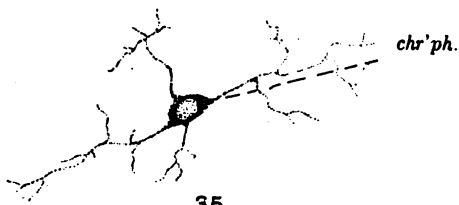
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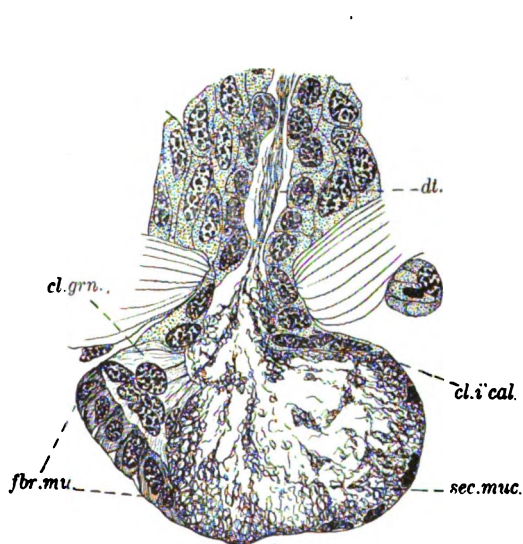
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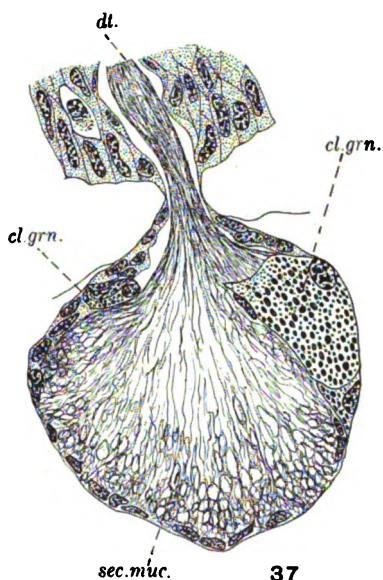
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37

Resumen por la autora, Caroline B. Thompson.
Wellesley College y U. S. Bureau of Entomology.

La "tercera forma," tipo reproductor áptero de los termitas.

Los termitas *Reticulitermes flavipes*, *R. virginicus* y *Prorhinotermes simplex* presentan cinco castas distintas, tres reproductoras y dos estériles: 1. La primera forma, con alas largas. 2. Segunda forma, con vestigios de alas. 3. Tercera forma, áptera. 4. Obreros, y 5. Soldados. Los adultos completamente desarrollados de *R. flavipes* se conocían desde 1915; los adultos jóvenes y las ninfas se describen por primera vez en el presente trabajo. Los individuos de la tercera forma se consideraron como raros en un principio; los autores han encontrado que no son poco comunes, sino que han pasado desapercibidos a causa de su semejanza con los obreros. Los adultos jóvenes de la tercera forma y las ninfas de *R. flavipes* y *R. virginicus* se distinguen de los obreros por el color de la cabeza, número de segmentos antenales, tamaño de los ojos compuestos, forma y color del abdomen y por muchos caracteres internos, como por ejemplo, los órganos sexuales funcionales y no funcionales, cerebro, cantidad de tejido adiposo, etc.

Semejantes diferencias distinguen a los sub-adultos de la tercera forma y ninfas del termita de Florida, *Prorhinotermes simplex*. En esta especie los individuos de la tercera forma son muy comunes.

Las cinco castas de las tres especies de termitas mencionadas forman una serie con correlación de tamaño y grado de desarrollo del cerebro, ojos y órganos sexuales, y en general hay una gradación en el tamaño de estos órganos a partir de la primera forma hasta el obrero y soldado. Los autores creen que la existencia de castas puede explicarse mejor como mutaciones ancestrales del tipo alado.

Translation by Josef F. Nonidez
Cornell Medical College, New York

THE 'THIRD FORM,' THE WINGLESS REPRODUCTIVE TYPE OF TERMITES: RETICULITERMES AND PRORHINOTERMES

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AND

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TWENTY FIGURES AND THREE PLATES (FIGURES TWENTY-ONE TO THIRTY-EIGHT)

INTRODUCTION

The most obvious character by which the various reproductive castes of termites may be recognized is the presence or absence of wings or wing vestiges. In *Reticulitermes flavipes* Kol., the common termite of the northeastern United States, there are three reproductive castes, or types, which are distinguishable by: 1) the long wings, possessed during the swarming period and afterward remaining only as short stubs, the so-called 'scales;' 2) the short scale-like wing pads, vestiges of wings; 3) the absence of either wings or wing pads. The two sterile castes of this termite are wholly wingless, and are: 4) the worker, recognized also by its grayish abdomen, and, 5) the soldier, with its elongated head. These five castes occur in most termites, with exceptions according to the family or genus, and all castes contain both sexes.

Of the reproductive castes the winged forms were best known to the earlier naturalists, being more conspicuous on account of their long wings and habit of swarming, their greater size, and darker pigmentation. The smaller lighter colored forms with wing vestiges, or without either wings or wing vestiges, lived underground or deep within their galleries in wood, and were less well known or understood. The greater importance attrib-

uted to the winged forms is shown by the names given them by eighteenth- and nineteenth-century observers, such as 'perfect insects,' 'true' queens and kings, 'royal pairs,' etc. They were supposed to be the founders and progenitors of all colonies, while the less conspicuous forms with wing vestiges or entirely wingless, termed the 'substitute' or 'complemental' forms, were assigned the rôle of understudy, and were supposed to be waiting in a semideveloped condition, how patiently or impatiently we are not told, for some chance or disaster to remove one of the 'royal pair,' whose place would then be filled from their ranks. Grassi ('93-'94) even went so far as to advance the hypothesis that these different reproductive castes, and also the sterile workers and soldiers, could be produced at the will of the colony by some unknown extrinsic means such as feeding, care, parasitic action, etc.

More recent work¹ has shown that the fertile and sterile types as nymphs are internally differentiated at the time of hatching and that very early in their postembryonic development all the adult castes may be distinguished. This, of course, proves that the cause of the different types is of intrinsic or germinal origin, and disproves Grassi's hypothesis of determining or changing the castes of termites by external means.

As far back as the time of Lespès ('56) the nymphs of the two reproductive castes with long wings and short wing vestiges were known and described. These nymphs possessed long wing pads and short wing pads, respectively, and were termed by Lespès, 'nymphs of the first form,' and 'nymphs of the second form.' Lespès either was not acquainted with or did not refer to the nymphs of the wingless reproductive form.

Grassi ('93-'94) recognized, in addition to the 'true' or 'perfect insects' with long wings, the two reproductive forms with short scaly wing vestiges and without any vestiges, but in reading Grassi one gets the impression that the distinction between these so-called 'substitute' and 'complemental' forms was vague, or at least seemed unimportant. The main point was that they should be ready to replace the 'royal forms' in case of need.

¹ Bugnion, '12, '13; Thompson, '17, '19.

In regard to the developing nymphs, Grassi recognized Lespès' 'nymphs of the first form' and 'nymphs of the second form,' but evidently believed that, taken young enough and with proper treatment, any nymph might be changed into anything else.

In order to simplify the rather confused and at present misleading nomenclature of Grassi and others, one of the writers (Thompson, '17) has proposed that the three reproductive castes, with long wings, short wing vestiges, and no wings, should be termed, respectively, in conformity with Lespès' terminology for nymphs, adults of the first, second, and third form. This terminology will be used in the present paper.

It was also proposed that the nymphs of the wingless reproductive form of *R. flavipes*, which were then unknown, should, if discovered later, be termed nymphs of the third form. These nymphs have since been found by one of the writers (T. E. S.), and have been studied and positively identified as such by the other writer (C. B. T.).

When the third-form queens of *R. flavipes* and *R. virginicus* Banks were first found and described (Snyder, '15), and for some time after, they were considered as of rare or infrequent occurrence, but so many specimens have been collected recently by one of the writers (T. E. S.) that it may be stated with certainty that the third forms of *Reticulitermes flavipes*, *virginicus*, and *tibialis* Banks are not uncommon.

A full account of the morphology of the nearly mature nymph and adult of the third form of *R. flavipes* will be given below. The close external resemblance to workers shown by these partly mature third-form nymphs at first suggested that the third form might be merely a fertile worker, but after careful study, this view has been abandoned in favor of the opinion that the third form of *Reticulitermes* is a distinct morphological caste.

To reach this opinion a careful comparison has been made of the third form of *R. flavipes* with the other castes of this species. Similar studies were made with species in four other genera of termites: the Antillean termite, *Protrichotermes simplex* Hagen,² *Termopsis* from the Pacific coast, *Neotermes* and *Kaloterme*s

² Formerly *Arrhinotermes simplex*. See Banks and Snyder, '19.

from Florida, in all of which the third form is present, and is of frequent occurrence in species of the first two genera.

Comparative studies of the castes of *Reticulitermes* and *Prorhinotermes* form the subject matter of the present paper, and it will readily be seen that, although the elucidation of the third form is the chief end in view, it is necessary for comparison to describe or figure the various castes of each genus.

Two morphological facts, which apply to all the termites here described, should be borne in mind: first, that, together with certain external characters, such as wings, there is a correlation in the size, structure, and degree of development of the brain, the eyes, and the sex organs; second, that, in general, in whatever castes are represented, there is a gradation in the size of these organs, from the first form down to the worker or soldier.

One writer (T. E. S.) is the discoverer of the third-form nymphs and adults, and is responsible for the collection of most of the material and for the biological data of this paper, the other writer (C. B. T.) is accountable for the morphological study, the drawings, and for a part of the *Termopsis* material.

RETICULITERMES FLAVIPES KOLLAR

This species is widely distributed throughout the eastern United States from Maine to the Florida Everglades. It is essentially a wood destroyer, but colonies may be found either in decaying wood or in the ground. Throughout the world, wherever they occur, the species of this genus are most destructive to timber and the woodwork of buildings.

The castes of *R. flavipes* are five in number, three of which are reproductive or fertile forms, while two are non-reproductive or sterile. The reproductive castes are: 1) the first form, with long wings at the time of swarming, and mere stubs, the 'scales,' after deâlation; 2) the second form, with short scaly wing vestiges; 3) the third form, wingless, with creamy white abdomen. The sterile castes are: 4) the worker, wingless, with grayish abdomen, and, 5) the soldier, wingless, with elongated head. All of these castes are found in both the nymphal and adult conditions, according to the season of the year. In a normal colony in which

the parents are first-form adults the nymphs of all five castes may be found.*

R. flavipes is one of the smaller termites, the smallest adults, the workers, measuring only 5.5 mm. in length, the soldiers from 6 to 7 mm., and the winged first-forms, not including the wings, 6 mm. After mating has occurred, the abdomens of the reproductive forms undergo the postadult growth, characteristic of all termites, and which is particularly marked in this genus, although less so than in many tropical genera. The first-form adults (queens) increase from a body length of 6 mm. at the time of maturity up to 14.5 mm., the second forms from 6 mm. to 12 mm., the third forms from 6 mm. to 9 mm. The size increase, although marked, is considerably less in males. Some loss of activity in the queens accompanies the increase in bulk.

Development. The eggs are 0.6 to 0.7 mm. long, the newly hatched nymphs are 1.1 mm. in length, and although externally all alike, they are differentiated internally into two types: a) with large brain and large sex organs, giving rise to the reproductive castes, and, b) with small brain and small sex organs, giving rise to the worker and the soldier. More exact data than we possess at present upon the molting and the later phases of development of this and other species are greatly needed.

The first form of R. flavipes

The first form has three well-defined phases of development: a) the nymphs of the first form, with long wing pads, creamy white body, and light brown or pinkish eyes; b) the winged adults of the first form, with long wings, dark brown body, and black eyes, body length 6 mm., length to tip of wings 9 to 10 mm.; c) the older males and females of the first form, with enlarged abdomen, and the scales of the shed wings, length 6 to 14.5 mm.

The nymph of the first form. Very soon after hatching (body length, 1.3 to 1.4 mm.) it may be seen that among the reproductive nymphs with large brain and large sex organs, there are

* For data concerning colonies in which the parents are second-form or third-form adults see Thompson and Snyder, '19.

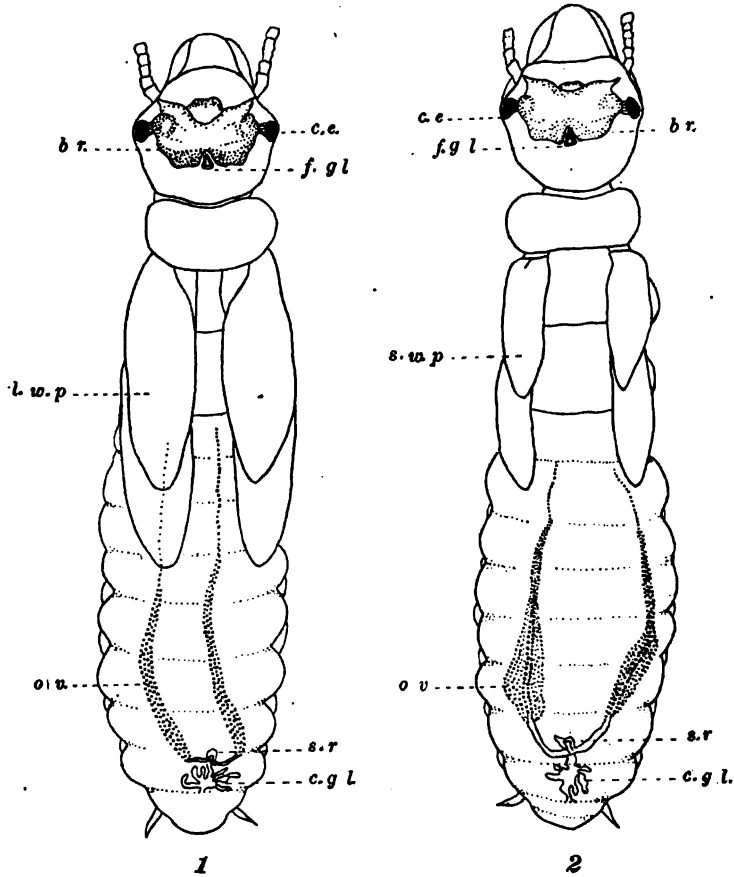


Fig. 1 *Reticulitermes flavipes*. Nymph of the first form, female, nearly mature, drawn from a whole mount.

Fig. 2 *Reticulitermes flavipes*. Nymph of the second form, female, nearly mature, drawn from a whole mount. The ovaries, *ov*, which in their adult development are smaller than those of the first-form female, are here larger, owing to the earlier maturity of the ova of this nymph. *br*, brain; *ce*, compound eyes; *c.gl*, colleterial gland; *f.gl*, frontal gland; *l.w.p*, long wing pad; *s.w.p*, short wing pad; *s.r*, seminal receptacle; *ov*, ovary. Spencer oc. 6, obj. 32 mm., stage level. Reduced one-third.

two fairly distinct types, one with brain and sex organs of normal size, nymphs of the first form; the other with these organs slightly smaller, nymphs of the second form. The longer and shorter wing pads of the two forms are first visible when the nymphs have attained a body length of about 4 mm.

The characteristic external features of a nearly mature nymph of the first form, about 6 mm. long (fig. 1), are the rounded head, tapering slightly toward the posterior end, the prominent light brownish or pinkish compound eyes, and the long wing pads which extend backward as far as the fifth abdominal segment. The number of antennal segments is eighteen. The abdomen is more slender than in the second-form nymph of similar age, the same is true of the legs. On the ventral surface of the ninth abdominal segment, in both sexes, two genital appendices are present.

Internally, as seen from stained whole mounts and sections, the brain is the largest of any of the castes, with the most highly developed mushroom bodies, and the largest optic lobes in correlation with the largest compound eyes. The frontal gland is also the largest and is acquiring a glandular structure (Thompson, '16). The venation of the long slender wing pads is similar to that of the adult. The abdomen is slightly swollen, the sex organs are fully formed, but are not as near the functioning period as those of the second-form nymph. In the female (figs. 1, 21), the ovaries are long and slender, extending the entire length of the abdomen, but the contained ova are not yet greatly enlarged. The seminal receptacle is fully formed, the oviducts and the vaginal duct are united and have a visible lumen. The colleterial gland is large and convoluted. In the male (fig. 24), the testes are large, with many lobes, the vasa deferentia have a lumen, and the seminal vesicles are elongated and convoluted. Posterior to their junction with the seminal vesicles the vasa deferentia continue as a single ejaculatory duct which ends in a muscular and evidently protrusible penis, enclosed in a sheath. As in all reproductive forms the space between the epidermal layer and the body organs is filled with masses of fatty tissue.

The winged adult of the first form. The dark brown body, 6 mm. long, and very similar in form to that of the nymph of the

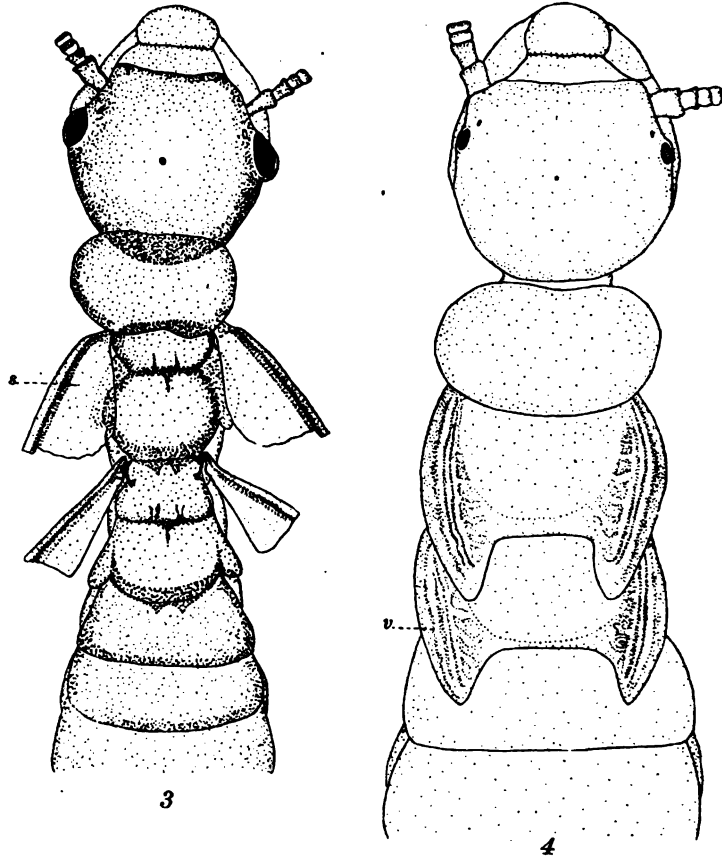


Fig. 3 *Reticulitermes flavipes*. Young dealated adult of the first form, surface view. The 'scales,' *s*, of the long wings are present.

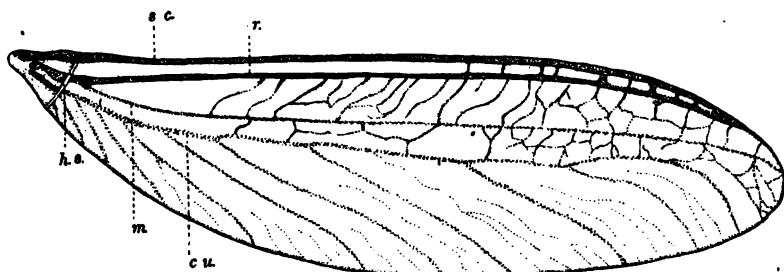
Fig. 4 *Reticulitermes flavipes*. Older enlarged adult of the second form, surface view. The vestiges of the wings, *v*, have an indistinct venation similar to that of the wings of the first form. Spencer oc. 6, obj. 32 mm., table level, reduced one-third.

first form, the black pigment of the compound eyes, and the long filmy wings are the chief external characters of the winged first-form adults. Two minor differences are the increased size of the

sternite of the seventh abdominal segment and the absence of the genital appendices (styles) on the ninth abdominal segment in first-form adult females, these small appendices (styles) having disappeared with the last molted skin (Snyder, '15, p. 40).

The wings of the adult of *R. flavipes* (figs. 3, 5) are dissimilar in size, the mesothoracic wings being slightly larger, and have slight differences in venation.

According to both Comstock ('18) and Holmgren ('09), four veins are present: the subcosta, *sc*, which forms the costal margin of the wing, and, according to Comstock, "is greatly thickened. It is probably formed by the coalescence of costa and subcosta,



5

Fig. 5 *Reticulitermes flavipes*. Hind wing of first-form adult. *sc*, subcosta; *r*, radius; *m*, media; *cu*, cubitus; *h.s.*, humeral suture. Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

and may also include vein $R\ 2 + 3$." Parallel to the subcosta and also chitinized is the radius, *r*; the media, *m*, is delicate, with but little chitin, and the same is true of the cubitus, *cu*, with its many branches along the anal margin of the wing. The chief differences in the venation of the two wings are as follows: fore wings—1) the larger 'scale,' i.e., the proximal part of the wing which remains attached to the thorax after deälation, and which is bounded distally by the 'humeral suture,' the line of breakage; 2) the media separate from the radius near their origin, within the 'scale;' 3) no 'radial sector,' or distinct branch of the radius, is present; hind wings—1) the smaller 'scale' and indistinct humeral suture; 2) the radius and media are coalesced within the area of the 'scale,' but separate immediately after leaving it; 3) in some specimens a faint 'radial sector' is present.

After swarming is over, mates are chosen, the wings are shed, breaking along the lines of the 'humeral suture,' and the subterranean life begins. The sex organs mature and in time become the largest of any caste.

The enlarged adults of the first form. After a period of time, probably within two years, the abdomen enlarges in those first-form adults which have mated, and its size then increases with the age of the individual, but especially so in females. As the abdomen grows the color of the chitinized dorsal and ventral plates becomes a light golden brown, and the non-chitinized unpigmented portions increase enormously, so that in the oldest males and females the abdomen is a huge whitish sac with nine pairs of small yellowish plates indicating the former segments. The body length of these enlarged adults ranges from 7 to 14.5 mm. Sections show that the space within the abdomen of the female is chiefly filled by the enormously increased ovaries, and by oenocytes, glandular, and fatty tissue. The muscles, especially the jaw muscles in the head, degenerate and their place is filled by fatty tissue. This degeneration of the jaw muscles is due to the fact that the reproductive forms are now fed by the workers on partly digested food and no longer masticate wood as they were compelled to do before the first broods of workers were raised. Mature females of the first, second, or third forms do not survive in captivity unless enough workers are present to feed them.

The second form of R. flavipes

The second form, like the first, is found in three phases of development: a) the nymphs of the second form, with short wing pads, and colorless body and eyes; b) the young adults of the second form, with short scaly wing vestiges, and straw colored or grayish body 6 to 7 mm.; c) the older adults of the second form, with wing vestiges, and enlarged abdomen 7 to 12 mm

The nymph of the second form. Soon after hatching, as stated above, the young nymphs of the second form may be distinguished microscopically from those of the first form by their slightly smaller brain and sex organs. When they have attained a body length of 4 mm. the shorter wing pads may be recognized.

In the nearly mature second-form nymph of 6 mm. (fig. 2) the distinguishing external features are the nearly colorless compound eyes, the shorter scale-like wing pads, extending backward only as far as the third abdominal segment, and the rather stout abdomen. The legs are slightly heavier, notably the femora and the tibiae, than in the first-form nymphs. The number of antennal segments is eighteen. In both sexes genital appendices are present on the ninth abdominal segment.

The venation of the short wing pads is similar to that found in the wing vestiges of the second form adult (fig. 4), and also to that of the first form.

Internally, the chief difference between first- and second-form nymphs is in the size of the organs. In the nymph of the second form the brain and its main parts are on a slightly smaller scale, likewise the compound eyes, which also contain less pigment, and the frontal gland.

The reproductive organs appear larger in the nearly mature second-form nymph than in the first (figs. 1, 2, 21, 22). In the female nymph of the second form the bulk of the ovaries is greater and the contained ova are larger, the oviduct has a greater width, and the seminal receptacle is nearly twice the size of similar organs in the first-form nymph. The colleterial gland also is larger in the second-form nymph.

In the male nymph of the second form (fig. 25), the testes are more than double the size of those of the first form, the vasa deferentia are wider and more twisted, and the length and convolutions of the seminal vesicles greater.

This is apparently not a morphological difference, but a physiological one, due to the earlier maturity of the sex cells of second-form individuals. Sections of the abdomens of females of the three reproductive forms, in specimens of nearly maximum distention, show that the space filled by the egg tubes, and probably the number of ova, is greatest in the first form, less in the second form, and least in the third form (Thompson and Snyder, '19, figs. 6 to 12). The difference in the mating habits of the first two forms is evidently correlated with the times of maturity of their reproductive organs. The first-form adults swarm,

taking longer or shorter aerial flights, and drop to the ground; then, after shedding their wings and selecting their mates, the search for a new habitation may consume some time. The second-form adults do not swarm and possibly need less time for the establishment of the new habitation before egg laying begins. It should, however, be stated that it is not known with certainty how the second-form adults establish new colonies.

The young adults of the second form. The yellowish color of the body, the small light-brown compound eyes rimmed with white, and the thin scale-like appearance of the wing vestiges distinguish the second-form adults externally. Since the wing pads, or vestiges, cease their growth sooner than the abdominal segments, the former are relatively shorter in the adult second form than in the nymph, sometimes extending only to the beginning of the second abdominal segment. Genital appendices are absent from the ninth segment of the female. The sides of the abdomen of young second-form adults, especially of the males, are usually laterally compressed, giving the specimen a ridged appearance.

Internally the chief differences between first- and second-form adults are quantitative, the second form having a smaller brain, compound eyes, frontal gland, and sex organs.

The venation of the wing vestiges of the second-form adult and the wing pads of the second-form nymph is the same and homologous with that of the first-form adult and nymph. The rudiments of the four veins, subcosta, *sc.*, radius, *r*, media, *m*, and cubitus, *cu*, may be noted in figure 4, and one of the differences between fore and hind wings, mentioned above, namely, the coalescence of the radius and media in the proximal part of the hind wing, is also here present. There are no traces of the humeral suture in either wing vestige, and no signs of even a faint 'radial sector' in the hind vestige.

The enlarged adults of the second form. These forms may attain a body length of 12 mm. in living specimens, but are never as long or as stout as those of the first form. The body color is yellowish rather than creamy white, with darker chitinated areas, as in the first forms. Very often the abdomen of the

enlarged second-form female is irregular or lumpy in outline, perhaps due to irregularities in the development of the sex organs or fat bodies. The internal changes are similar to those noted for the first form: increase in size of the sex organs and the fatty and glandular tissues, and degeneration of most body muscles.

The third form of R. flavipes

The third form of *R. flavipes* has been found in the same three phases as the nymphs of the first and second form, namely:

a) the nymphs of the third form, wingless, with pure white head and body, and eyes that are invisible in the living or unstained specimen; b) the young adults of the third form, wingless, head and body pure white, opaque and not transparent, about 6 mm. long; c) the older adults of the third form with enlarged abdomen, wingless, head and body white, 7 to 9 mm. long.

The enlarged adults of the third form of *R. flavipes* and *R. virginicus* have been known for several years (Snyder, '15, '16); the nymphs and the young third-form adults of *R. flavipes* have been only recently recognized as such, and are here described for the first time. The close resemblance of the living nymphs and young adults of the third form to the workers of their species is evidently the reason why this caste of *Reticulitermes*, except in the enlarged adult phase, has hitherto been overlooked.

For many years, one of the writers (T. E. S.) had noted that in certain colonies of species of *Reticulitermes* where there were numerous eggs and recently hatched young it was impossible to find any enlarged first, second, or even third reproductive forms. Nevertheless, in these colonies nymphs, worker-like in form but with creamy white abdomens, often occurred. It was then suspected that these might be young reproductive forms of the third type.

Only after careful study have criteria been found for distinguishing the worker and third-form castes in *R. flavipes* and also in the related southern species *R. virginicus*. Since these criteria were established it is interesting to note that in looking over old

material collected several years ago, and in examining stained slides, one writer (C. B. T.) has found that both third-form nymphs and young adults are present among forms that were supposed to be workers. The strong superficial resemblance

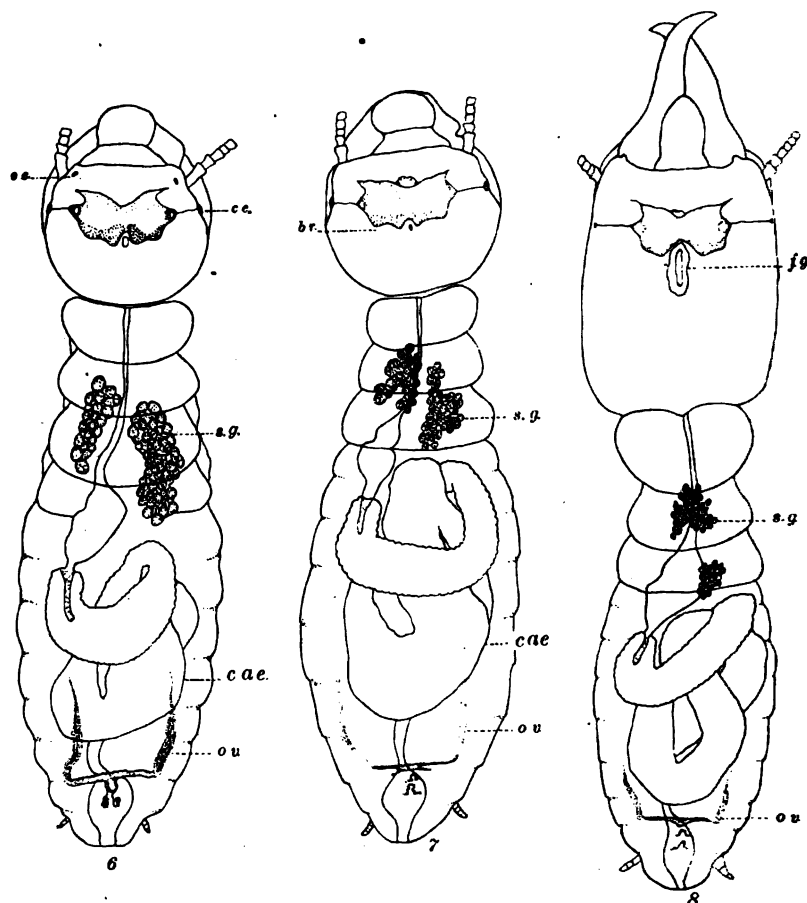


Fig. 6 *Reticulitermes flavipes*. Third-form nymph, female, nearly mature, drawn from a whole mount.

Fig. 7 *Reticulitermes flavipes*. Worker, female, adult, drawn from a whole mount.

Fig. 8 *Reticulitermes flavipes*. Soldier, female, adult, drawn from a whole mount. *Br*, brain; *cae*, caecum; *c.e.*, compound eye; *f.g.*, frontal gland; *oc*, ocellus; *ov*, ovary; *s.g.*, salivary gland. Spencer oc. 6, obj. 32 mm., table level, reduced one-half.

between these two castes suggested at first, as stated in the introduction, that the third-form adult might be merely a physiological phase of the worker caste or, in other words, a fertile worker. The infrequent occurrence of the third-form adults might indicate, it was thought, that occasionally a worker nymph would revert to its probable ancestral condition of

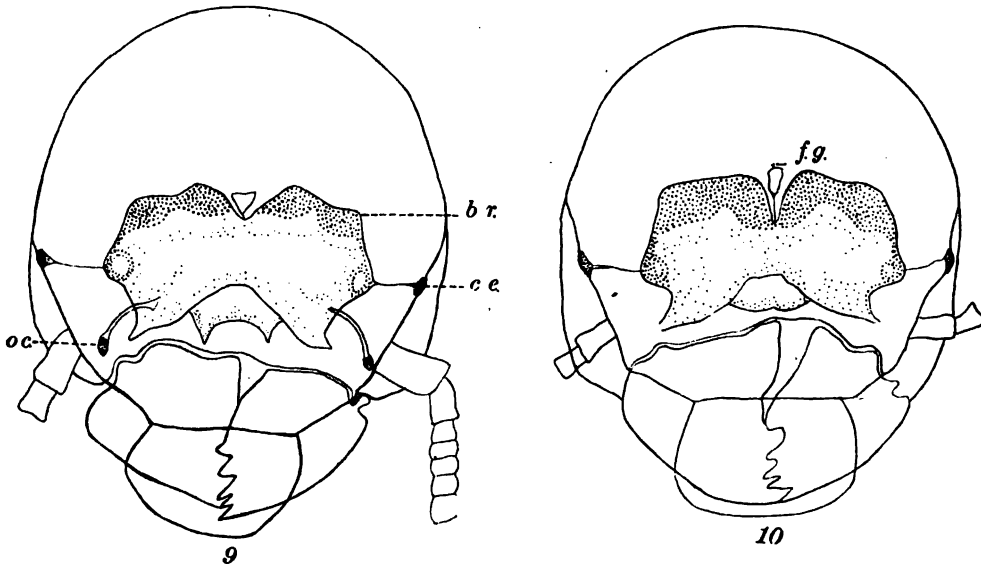


Fig. 9 *Reticulitermes flavipes*. Third-form adult, female, optical section of head.

Fig. 10 *Reticulitermes flavipes*. Worker, adult, optical section of head. *br*, brain; *c.e.*, compound eye; *f.g.*, frontal gland; *oc*, ocellus. Spencer oc. 6, obj. 16 mm., table level, reduced one-third.

fertility, as in ants and bees, developing into the rare and fertile third-form adult; the majority of the worker nymphs, however, developing into sterile workers. A careful comparison of the two castes in question proves beyond doubt that there are here two distinct castes, biologically, morphologically, and physiologically different, and that the superficial resemblance is more than counterbalanced by the many minute structural differences revealed by the study of whole mounts and sections.

The early development of the third-form nymphs (under 4 mm.) has not been traced. Their resemblance to the nymphs of workers is so close that it would be very difficult to recognize one from the other in early stages in which the adult characters necessary to distinguish them have not yet appeared. To illustrate, three important adult characters which separate the third form from the worker are: the number of antennal segments, the thickness of the head chitin, and the fusion or non-fusion of the three embryonic fundaments of the female reproductive system. It is obvious that these cannot be used in studying young nymphs in all of which the antennal segments are incomplete, the chitin thin, and the parts of the reproductive system separate. Further detailed study, however, may develop means for distinguishing the very youngest nymphs of these two castes.

The nymph of the third form. The nearly mature nymph of the third form, 5 to 6 mm. long (fig. 6), has a pure white head and body, a thorax devoid of either wings or wing pads, seventeen or eighteen antennal segments, and compound eyes that are so small and contain so little pigment that they are practically invisible in the living or unstained specimen, and are seen only with the microscope after staining. Genital appendices are present on the ninth abdominal segment in both sexes. These characters will serve to distinguish the third-form nymph from those of the first and second form.

The third-form nymph of 5 to 6 mm. may be distinguished by the following characters from the adult worker, 5.5 mm. (fig. 7), which, it will be recalled, is also wingless, using for this comparison unstained specimens and a low-power lens: 1) the color of the head—third-form nymph: white, worker: yellow, due to the thicker chitin; 2) the color and general appearance of the abdomen—third-form nymph: opaque or dense creamy white, firm texture, long, tapering, intestinal contents not woody and not visible, worker: transparent or glistening white, loose texture, shorter, blunt, intestinal contents woody, and visible as a dark mass; 3) the number of antennal segments—third-form nymph: seventeen to eighteen, worker: sixteen. See table 1.

In all three reproductive forms the dense creamy white or opaque abdomen is in marked contrast to the partly transparent

abdomen of the workers and soldiers. In sections of the abdomens of the different castes the cause is clearly seen. The spaces of the body cavity between the viscera are filled, in all three reproductive castes, by dense masses of fat cells; in the sterile forms there are fewer of these masses of fatty tissue and many more spaces. This also accounts for the firm texture and outline noted for the abdomen of the reproductive forms and for its looser texture in the worker and soldier.

In third-form nymphs with a body length of 4.5 mm. or less the three fundamentals of the female reproductive system—ovary and oviducts, seminal receptacle and proximal part of the vaginal duct, colleterial gland and distal part of the vaginal duct—are found as separate parts, not yet fused, an embryonic condition characteristic of all young nymphs and of adult workers and soldiers (Knower, '01) (figs. 27, 28).

The parts of the female reproductive system of a third-form nymph of 5 to 5.5 mm. are completely fused although not yet of full size. The ovaries (fig. 23) contain eggs which show signs of growth, the seminal receptacle is large, and the vaginal duct has a lumen. The colleterial gland is small, though convoluted. In the male (fig. 26) the testes are large and lobed, in size surpassing those of the first form, the vasa deferentia and the seminal vesicles are convoluted, and the muscular penis is well developed. Here is another important distinction between the third form and the worker, the former with fully developed and functional sex organs (figs. 6, 23, 26), the latter with these organs in a vestigial and non-functional condition (figs. 7, 27, 29).

The contents of the alimentary canal furnish another difference between the two castes. The third form, like the other reproductive castes, is evidently fed on predigested food furnished by the worker and does not masticate wood, for the large intestinal sac, the 'caecum,' is always clear and free from the dark woody masses found in the worker. The parts of the digestive system differ slightly in size in the different castes, and the salivary glands are larger in the reproductive forms than in the two sterile castes.

The young adult of the third form. The young adult differs from the nearly mature nymph just described chiefly in size and

in the maturity of the sex organs. Specimens which are mature, or nearly so, are 6 mm. in length. The very small compound eyes are not visible in living or unstained specimens, the thorax is entirely wingless, and the color of the body almost white. Lateral ocelli are prominent in stained specimens.

Investigation of the internal anatomy of the young third-form adult brings to light other characters which further distinguish this caste from the worker. The brain of the third-form adult (figs. 6, 9) is markedly smaller than that of the first or second form, but is slightly greater in bulk than in the worker (fig. 10), due to the greater number of cells in the mushroom bodies and optic lobes. The compound eyes are small and degenerate compared with those of the other reproductive forms, but, like their optic lobes, they are slightly larger than in the worker. Lateral ocelli, which are totally lacking in the worker, are present in the third form. The frontal gland is intermediate in size between that of the second form and of the worker; it is probably not glandular. The chitin covering the head is thin in all three reproductive castes; in the worker it is very thick and tough (figs. 31, 32). The mature sex organs are smaller than in either the first or second forms, though larger than those of the worker.

The older adults of the third form. These forms differ externally from the similar phases of the other two reproductive castes chiefly in the absence of wings, the white or pale yellow body, and the less distended abdomen. Genital appendices are present on the ninth abdominal segment of the male, but are not visible in the female. The body length is from 7 to 9 mm. These older third-form adults, on account of the less enlarged abdomen, never lose their activity; the legs, in correlation with this activity, are, like those of the active older second form, stouter and more muscular than the legs of the greatly distended and more sluggish first form. The jaw muscles have undergone a degeneration similar to that already noted in the older first and second forms. Fewer males than females have been collected, perhaps on account of their greater activity, but probably because they are more readily confused with workers.

The following table sets forth the chief structural differences between the third form and the worker of *R. flavipes*.

TABLE 1
Comparison of third form and worker
R. flavipes

	THIRD FORM	WORKER
Head:		
Color.....	White	Yellow
Chitin.....	Thin	Thick
Antennal segments.....	17-18	16
Compound eyes.....	Small	Slightly smaller
Lateral ocelli.....	Present	Absent
Frontal gland.....	Small	Vestigial
Brain.....	Small	Slightly smaller
Jaw muscles.....	Degenerate (adult)	Normal
Thorax.....	Wingless	Wingless
Salivary glands.....	Large	Smaller
Abdomen:		
Form.....	Slender	Blunt
Color.....	White, opaque	Transparent, showing intestinal contents
Distention.....	Enlarged in older forms	Not enlarged in older forms
Fatty tissue.....	Abundant	Very little
Genital appendices.....	Present in male; not visible in adult female	Present in both sexes
Reproductive organs....	Fully formed; functional	Embryonic; non-functional
Food.....	Probably fed by workers	Wood
Body length.....	6 mm. (young adult)	5.5 mm. (adult)

The third form of R. virginicus

This smaller southern species was, like the northern, at first known only in the enlarged adult phase, but in looking over unstained material and mounted specimens, both the nymphal and young adult phases have been detected in specimens that were formerly considered workers.

In figure 11, the head of an enlarged adult is shown, and in figure 12 the reproductive system of a young female nymph which is not yet mature. It will be noted that the ova have begun to enlarge, whereas the fusion of the three parts of the reproductive system, *ov.*, *s.r.*, *c.g.*, is not wholly completed. As a transition phase in development this specimen is especially interesting.

The worker of R. flavipes

The worker is found only in the nymphal and adult phases, since no postadult growth takes place as in the reproductive forms.

The development of the worker is a simpler process than that of the fertile castes and requires a shorter period of time—one year or less, instead of two. The newly hatched nymphs with the small brain and small sex organs, the 'worker-soldier' nymphs, give rise to both workers and soldiers, and in the early phases of development there are no external features to distinguish these two castes. With a body length of 3.75 mm. the two may be distinguished by internal features—size of the frontal gland (Thompson, '17)—but after a length of 4 to 4.5 mm. is attained, the worker and soldier are externally different.

The worker nymph differs from the adult worker only in size and a lighter body color.

The adult worker is 5.5 mm. long (fig. 7), the head is very similar in size to that of the third-form adult, but is yellowish, on account of the thick chitin (fig. 31). The compound eyes are small and practically invisible except with magnification. The thorax is wingless. The abdomen is rather blunt in outline, as compared with the reproductive forms, the skin appears thin and transparent, on account of the very few masses of fat cells in the body cavity and the many empty spaces, filled only by body fluids. The woody contents of the large intestinal sac, or caecum, show clearly through the skin, giving a grayish color to the abdomen, a good field mark for this worker. Genital appendices are present on the ninth abdominal segment of both sexes.

The internal structure. The brain (fig. 10), especially the optic lobes, and the compound eyes are greatly reduced in size; the frontal gland is a mere vestige, not glandular; the lateral ocelli are lacking. The reproductive organs are non-functional, having remained in an early embryonic stage of development. In the female, the three fundaments of this system have not undergone fusion, and the ovaries and ova are very minute (fig. 27). In

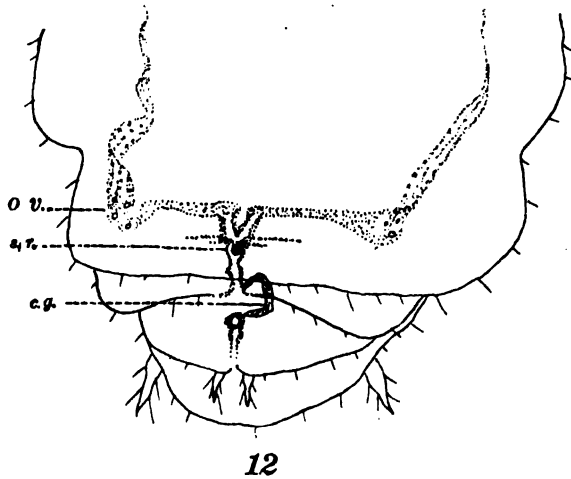
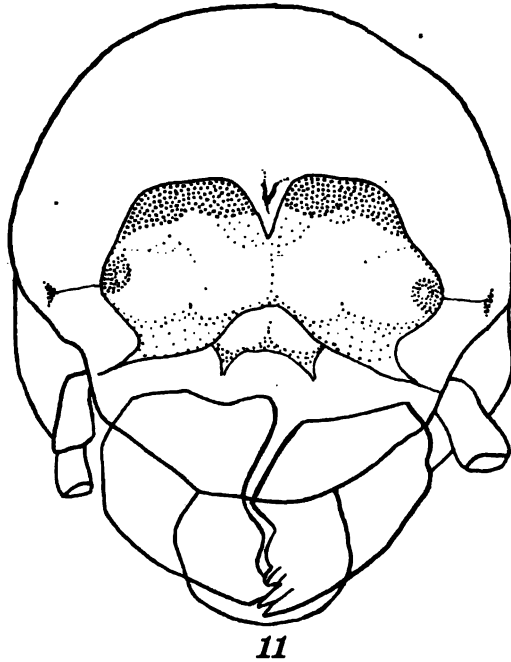


Fig. 11 *Reticulitermes virginicus*. Third-form adult, female, optical section of head.

Fig. 12 *Reticulitermes virginicus*. Third-form nymph, female, immature. *ov*, ovary; *s.r.*, seminal receptacle; *c.g.*, colleterial gland. The three parts of the reproductive system, though fused, are still embryonic in character. Spencer oc. 6, obj. 16 mm., stage level.

the male (fig. 29), the single ectodermal fundament, the future ejaculatory duct of the reproductive form, has fused with the mesodermal fundament of the seminal vesicles and testes, but growth has not proceeded beyond an embryonic phase.

The soldier of R. flavipes

The soldier, like the worker, is found only in the nymphal and adult phases

The early development is parallel with that of the worker, both castes existing side by side as worker-like nymphs with round heads until they are a little over 4 mm. long, at which period the soldier nymphs molt from their worker-like skins, emerging as young round-headed soldiers. The elongated heads are acquired after a later molt.

The adult soldier, 6 to 7 mm. long (fig. 8), has an elongated head covered with thick yellowish chitin. The mandibles are dark brown, and are long, slender, and curved, differing from the mandibles of the other castes. The vestigial compound eyes can be seen only with the microscope. The opening of the frontal gland is evident on the frontal surface of the head. The thorax is entirely wingless. The abdomen is rather short and flattened horizontally; it lacks the large fat masses of the reproductive castes and is consequently transparent. Genital appendices are present on the ninth abdominal segment of both sexes.

Internally (fig. 8), the brain and the compound eyes are the smallest of any caste, and lateral ocelli are lacking, the frontal gland is large and functional. In the female (fig. 28) the three fundaments of the reproductive system show the same lack of fusion as in the worker, again representing an embryonic stage of development. The ovaries are the smallest of any caste of this species. In the male (fig. 30) the two fundaments have fused, but the size of the organs indicates that they are not functional. The testes are small, the vasa deferentia straight and the seminal vesicles are not convoluted. An embryonic penis is present which was not noted in the male worker. The condition of the reproductive organs of both sexes indicates that the soldiers of *R. flavipes* are incapable of sexual reproduction.

The lack of woody contents in the intestinal sac, or caecum, may be interpreted that the soldiers are fed by the workers and do not masticate wood. This may also be inferred from the structure of the mandibles, which are not adapted to biting, and are used rather to threaten an approaching enemy. A familiar motion, when the soldier is approached by a pencil or needle, is to raise the mandibles and wave them defiantly.

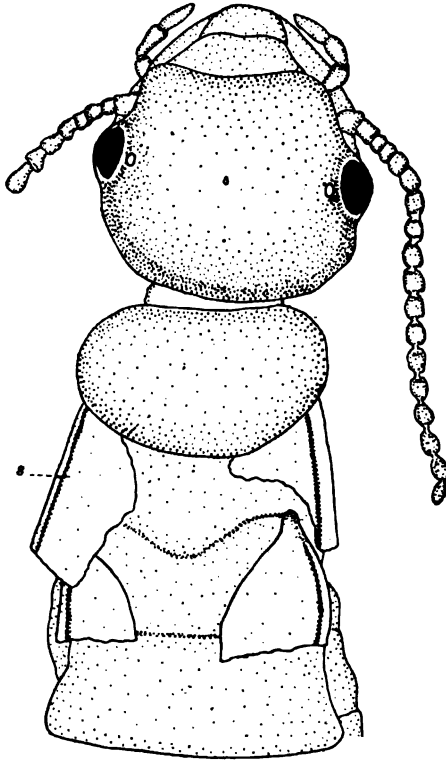
PRORHINOTERMES SIMPLEX HAGEN

In the United States this termite occurs only on the keys, along the sea coast, and in the interior of southern Florida; it is also found in Cuba and Jamaica. Its nests are in dead standing trees and in logs, but never in the ground. Considerable moisture is necessary for life, and this is also true of the species of *Reticulitermes* and *Termopsis*. *P. simplex* is injurious to piled lumber and to any timber which is kept moist by contact with the earth.

In the normal Florida colony of *P. simplex* four castes are of common occurrence, three of which have been collected in both the nymphal and adult conditions—the wingless third form, the worker, and the soldier—and one which is immature, a nymph of the second form with wing pads of very unusual shape. This nymph undoubtedly reaches maturity, but it has not yet been recognized in the adult state. One phase of a fifth caste, the dealated adult of the first form, is of fairly common occurrence in young colonies, but they have not yet been found in the winged phase, nor is there any record of the swarming of this species in Florida, possibly for lack of observations, since in near-by Cuba the first-form adults of *P. simplex* are known to swarm during the last of October. No first-form nymphs of *P. simplex* of the usual type, i.e., with long wing pads, have yet been found in Florida, but such nymphs are known in Jamaica. On the other hand, no adult second forms with fused wing pads, like those of the above-mentioned second-form nymph found in Florida, have been found in Cuba and Jamaica. A plausible explanation might be that the nymphs with peculiar fused wing pads are unusual first-form nymphs and later become the dealated adults, but a careful examination of these two forms shows that they belong without doubt to two different castes.

The first form of P. simplex

In Florida the first form of *P. simplex* has so far been found only in the deālated adult phase; in Jamaica nymphs of the first form with wing pads of normal shape occur, and in Cuba winged



13

Fig. 13 *Prorhinotermes simplex*. Deālated adult of the first form, surface view. S, 'scales' of the wings. Spencer oc. 6, obj. 32 mm., stage level, reduced one-third.

adults, and deālated adults have been found. The writers have examined specimens of the first form from Jamaica, but this material is unfortunately very scanty and in poor condition for histological study, so that only very meager details of this form can be given.

About all that can be said of the nymph of the first form is that the wing pads resemble those of a young first-form nymph of *Reticulitermes*, and are totally unlike the peculiar fused wing pads borne by the second-form nymph of *P. simplex*.

The winged adult of *P. simplex* has a body length of 5 mm., or 9 to 10 mm. to the tip of the wings. The general body color is a light reddish brown. The wings (fig. 14) have a venation quite similar to those of *R. flavipes* in that the two veins of the costal areas, subcosta, *sc*, and radius *r*, are chitinized, though those of the remainder of the wing are almost without chitin. The media is lacking in both wings, though possible traces of it may be recognized in the network of fine branches between

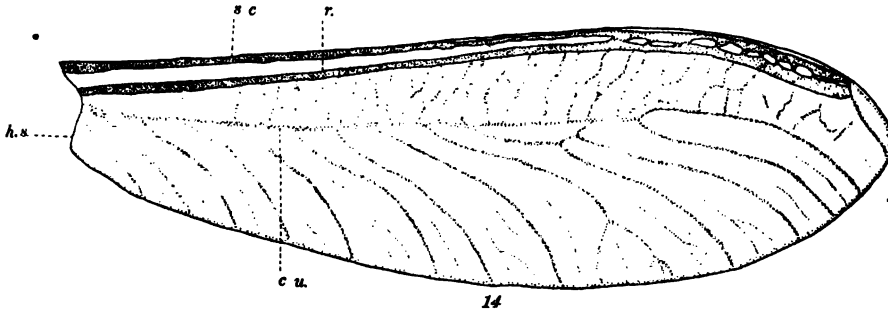
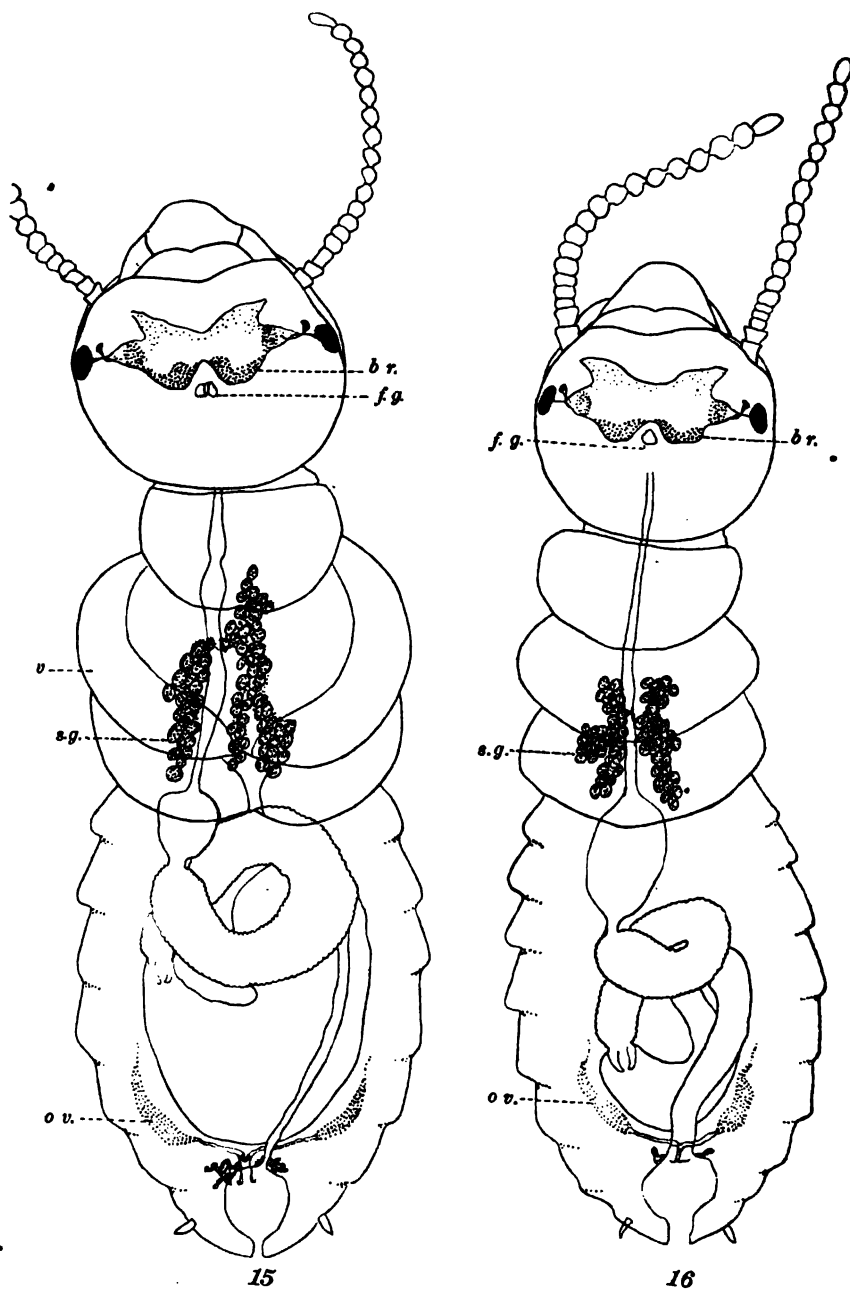


Fig. 14 *Prorhinotermes simplex*. Fore wing of first-form adult, broken off at the humeral suture, *h.s.* *sc*, subcosta; *r*, radius; *cu*, cubitus. No media is present. Spencer oc. 6, obj. 32 mm., stage level, reduced one-half.

the radius and cubitus, *cu*, especially in the hind wing. The two wings differ slightly in the venation, the presence of a 'radial sector' ($r\ 4 + 5$) in the hind wing and its absence in the fore wing being the chief difference.

The characters most worthy of note in the deälated adult of *P. simplex* (fig. 13), are the relatively small head and large compound eyes, the prominent opening of the frontal gland, the eighteen-jointed antennae, the 'scales' of the deälated wings, the two heavy veins of the costal margin still visible, and the enlarged abdomen. No sections or very successful stained whole mounts could be made with the available material, but it was seen that the brain is larger than that of the second-form nymph, and the same is true of the sex organs.



The second form of P. simplex

The second form of *P. simplex* has so far been found in Florida only in the nearly mature nymphal phase, and is not present in any phase in the Cuban or Jamaican material of this species. These nymphs (fig. 15) may be recognized by their peculiar wing pads, or vestigial wings, which differ greatly from those of the second type of other termites. The body length is 5.5 mm., less than that of the first-form adult, but slightly larger than either third-form nymphs or workers. The head is broader than that of the first-form adult, but the compound eyes are much smaller and less bulging. The abdomen is stouter than in either third-form nymph or worker. The body color both in life and after fixation is an opaque creamy white.

The number of antennal segments is quite constantly eighteen. The most characteristic features of the external anatomy are the fused wing pads (figs. 15, 19, 20), appearing as shield-shaped and partly transparent structures projecting beyond each lateral margin of the thorax and also in a posterior direction. The right and left wing pads of each segment are fused in the median line, and do not extend backward as in the normal type. The wing pads of the mesothoracic segment are larger than the metathoracic, and the venation differs slightly in the two. The same veins are present as in the wing of the first form, although the subcosta (figs. 19, 20, *sc*) is in a less developed stage, appearing in both wing pads merely as a thickened marginal area, except at the base of the hind wing where a true vein begins but shortly ends in the thickened margin. As in the first-form adult wing, a 'radial sector' ($r\ 4 + 5$) is present in the hind wing only.

Internal anatomy. The brain is more highly developed in the second-form nymph than in any of the other castes except the first form. The mushroom bodies and optic lobes are very large, and correlated with the size of the latter are the large

Fig. 15 *Prorhinotermes simplex*. Nymph of second form, female, drawn from a whole mount.

Fig. 16 *Prorhinotermes simplex*. Nymph of third form, female, drawn from a whole mount. *br*, brain; *f.g.*, frontal gland; *ov*, ovary; *s.g.*, salivary gland; *v*, vestige of wing. Leitz oc. 4, obj. 1, table level, reduced one-third.

deeply pigmented compound eyes. The lateral ocelli are also large, and in surface views such as that from which figure 15 has been drawn, the lateral ocellar nerves are clearly seen curving in toward the frontal surface of the brain. The frontal gland is present and is evidently of a glandular nature, but not yet functional. In some specimens it appears bilobed.

The reproductive system, although still immature, is larger than in any other caste of similar age.⁴ In the female (figs. 15, 33) the ovaries are large and stout and contain fairly large oblong ova; these again are larger than the ova of the third reproductive form. The oviducts are wide with an evident lumen, the vaginal duct is wide with convoluted inner surface, and is open to the exterior. The seminal receptacle is large and well developed, and the colleterial gland is greatly convoluted. This nymph fulfills the three criteria of fertility for a female termite: 1) the size of the ovaries; 2) the complete fusion of oviducts, vaginal ducts, and colleterial gland; 3) the size of the seminal receptacle. It will be seen below that some sterile females may fulfill one or even two of these requirements, but not all three.

In the male (fig. 37) the testes are of fair size and slightly lobed, and the seminal vesicles show signs of growth. The specimen shown in figure 37 gives evidence of being a young but fertile male.

The third form of P. simplex

The third form of *P. simplex* occurs in three phases: the nymphs, the young adult, and the enlarged adult. In Florida colonies, this form is very common and abundant.

The young and enlarged adults are easily recognized by the absence of wings, the size, 6 to 7 mm. long, the dark brown body pigment, and the distended abdomen. The young and nearly mature nymphs, however, are distinguished from workers with some difficulty, for there is here the same superficial resemblance as in the similar castes of *R. flavipes*, and, like these again, the many minute structural points of difference (table 2).

⁴ The Jamaican first-form nymphs examined were not in condition to show the finer details of structure of the reproductive system, so that they are not included in the present comparison.

The nearly mature third-form nymph of P. simplex. The nearly mature third-form nymph of 5 mm. (fig. 16) has the following external characters: head and body white, or the abdomen may appear gray like a worker, the chitin of the head thin, the number of antennal segments eighteen, the compound eyes are very

TABLE 2
Comparison of third form and worker
P. simplex

	THIRD FORM	WORKER
Head:		
Color.....	White	Yellowish
Chitin.....	Thin	Thick
Size.....	Medium sized	Small
Antennal segments.....	18	16
Compound eyes.....	Medium sized	Small
Lateral ocelli.....	Medium sized	Small
Frontal gland.....	Small, probably glandular	A bilobed ocellus
Brain.....	Medium sized	Small
Jaw muscles.....	Degenerate (adult)	Normal
Thorax.....	Wingless	Wingless
Thoracic plates.....	Large, posterior margins not indented	Small, posterior margins indented
Salivary glands.....	Large	Slightly smaller
Abdomen:		
Form.....	Slender	Swollen or blunt
Color.....	White, opaque, sometimes gray	Transparent, showing intestinal contents
Distention.....	Enlarged in older forms	Not enlarged in older forms
Fatty tissue.....	Abundant	Very little
Reproductive organs.....	Well developed, functional	Undeveloped, non-functional
Food.....	Wood, and probably also fed by workers	Wood
Body length.....	5.5 mm. (young adult)	5 mm. (adult)

slightly smaller and less pigmented than those of the second-form nymph, lateral ocelli are present, the thorax is entirely wingless, the thoracic plates are large and the posterior margins are convex and not indented in the median line, the abdomen is tapering, genital appendices are present on the ninth abdominal

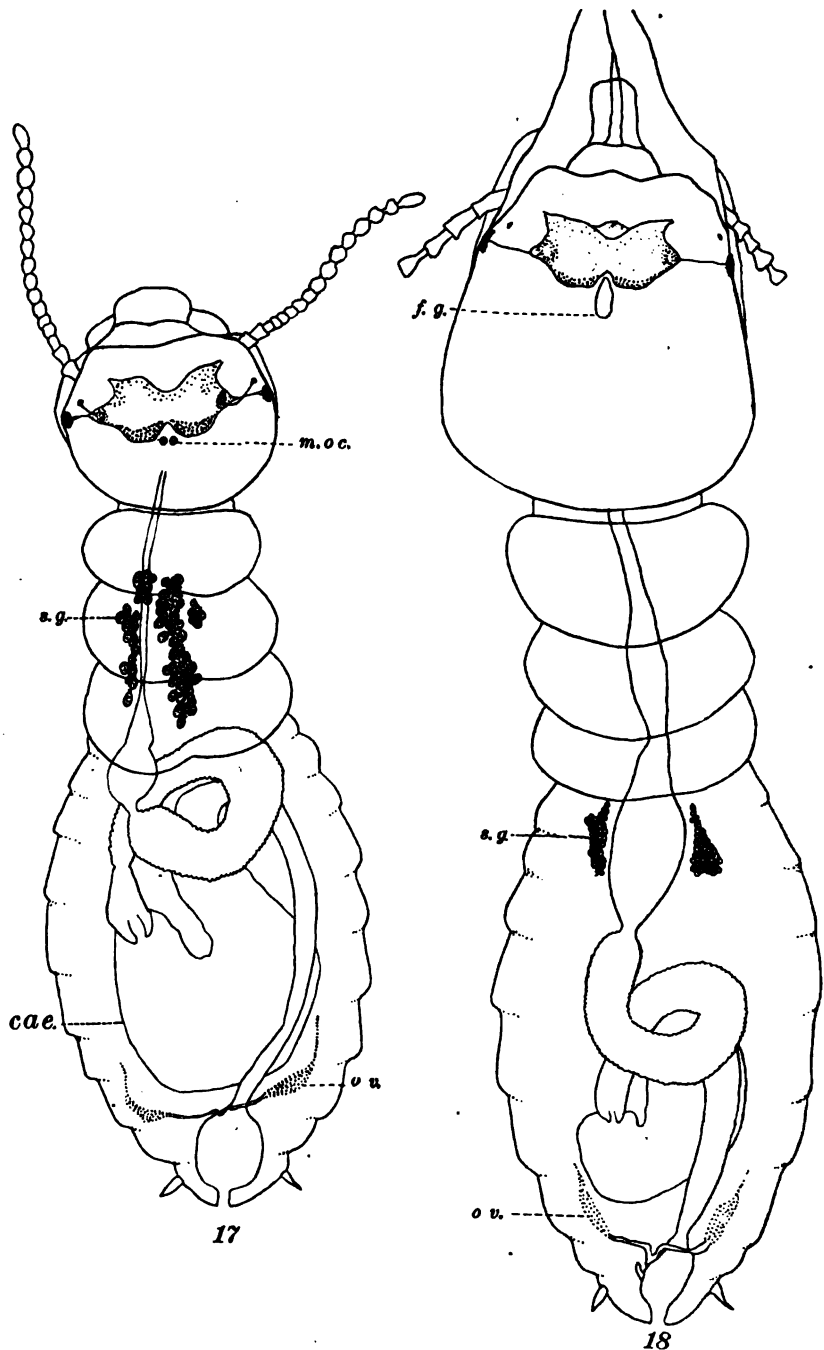


Fig. 17 *Prorhinotermes simplex*. Worker, female, drawn from a whole mount.

Fig. 18 *Prorhinotermes simplex*. Soldier, female, drawn from a whole mount. *cae.*, caecum; *f.g.*, frontal gland; *m.oc.*, median ocellus; *ov*, ovary; *s.g.*, salivary gland. Leitz oc. 4, obj. 1, table level, reduced one-third.

segment of both sexes. The gray color of the abdomen, due to the woody contents of the intestinal caecum, shows that for a time at least this caste feeds upon wood.

The following internal structures are, in comparison with the second-form nymph, on a slightly smaller scale: the brain, i.e., mushroom bodies and optic lobes, the compound eyes and ocelli, the frontal gland, and the sex organs (figs. 16, 34). The plan of all these organs is the same in the two castes, but in the third-form nymph each part is composed of fewer cells.

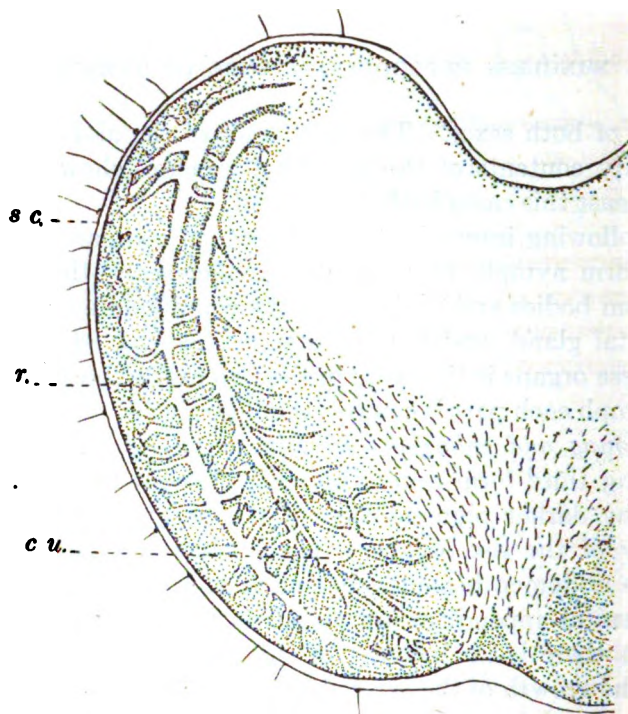
The young and enlarged third-form adults of P. simplex. In the young third-form adult the chief changes from the nymph are in the darker pigmentation of the body, the size, and the maturity of the sex organs. In the older adults of the third form the abdomen becomes distended and further changes take place; genital appendices are absent in the female. Within the abdomen there is an increase in the amount of fatty tissue as well as the growth of the sex cells. In the head the degeneration of the jaw muscles is accompanied by an increase in the masses of fat cells.

In the table on page 619, the chief structural differences between the third form and the worker of *P. simplex* are given.

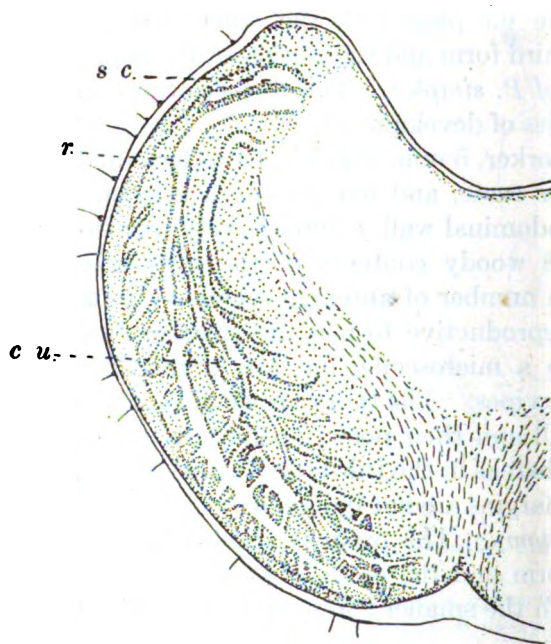
The worker of P. simplex. The worker occurs in the nymphal and adult phases of development.

The adult worker, 5 mm. (fig. 17), has a head and body smaller than any other caste, and has usually a grayish abdomen, the transparent abdominal wall, comparatively free from fat masses, permitting the woody contents of the intestinal sac to show through. The number of antennal segments is sixteen, two less than in the reproductive forms, the small compound eyes are seen best with a microscope, but are larger than those of the worker of *R. flavipes*. The thorax is wingless, the dorsal thoracic plates are small and their posterior margins are indented in the median line, whereas in the third form, these plates are larger and the posterior margins are not indented.

Internal anatomy. The brain is absolutely smaller than that of the third form and the optic lobes are relatively reduced, in correlation with the smaller compound eyes and the very minute



19



20

ocelli. In the place occupied by the frontal gland in other castes, a bilobed median ocellus occurs, an interesting reversion to the condition found in many insects, or a persistence of the embryonic phase of the frontal gland, Kochi ('00), Thompson ('16). The chitin of the head is thick, and since wood is habitually chewed the jaw muscles are normal and few fat masses occur in the head, nor in the abdomen.

The reproductive system fulfills some but not all of the conditions necessary for fertility, hence the worker of *P. simplex* is sterile, although approaching the probable ancestral condition of fertility. In the females (figs. 17, 35) the ovaries are smaller than in either the second or third forms, the contained ova are likewise smaller, but on the other hand, they are larger than in the workers of the related species, *R. flavipes* and *R. virginicus*. All three fundamentals of the reproductive system are completely fused and there is a continuous connection from ovaries to the exterior, although evidently without a lumen. The seminal receptacle is small and the colleterial gland not greatly convoluted. In the male, the fusion of the two fundamentals has occurred, but the growth of the parts, like those of the female, has been arrested, so that a non-functional condition is evident for both sexes.

The soldier of P. simplex. The soldier is found in the nymphal and adult phases of development. The adult soldier has a greater body length than any of the other castes, measuring 6.5 to 7 mm. (fig. 18). This is chiefly due to the large, elongated head. The head is wedge shaped, the broad surface at the posterior end, and darker in color than the body. The number of antennal segments in the specimens figured is nineteen, but this number frequently varies. The thoracic plates are larger than in the worker and the posterior margins are not indented in the median line.

Fig. 19 *Prorhinotermes simplex*. Mesothoracic wing vestige of nymph of the second form.

Fig. 20 *Prorhinotermes simplex*. Metathoracic wing vestige of nymph of the second form. The venation is similar to that of the wing of the first-form adult. *sc*, subcosta; *r*, radius; *cu*, cubitus. No media is present. Spencer oc. 6, obj. 16, stage level.

Internal anatomy. The brain (fig. 18) has a slightly greater bulk than in the worker, although the mushroom bodies are smaller. The optic lobes and the correlated compound eyes are both a little larger in this soldier than in its worker, a rather unusual occurrence. The lateral ocelli, though very small, are clearly visible, recalling the lateral ocelli of the soldier of *Kalotermes*. The frontal gland is large, glandular, and functional.

The reproductive system. In the male (fig. 38) all of the reproductive organs are present and fully fused, but the size of the organs indicates that their growth was arrested before development was complete, so that the male soldier of *P. simplex* is non-functional or sterile.

The female reproductive system (figs. 18, 36) is considerably larger in the soldier than in the worker, but, like that of the worker, is evidently non-functional or sterile, although a certain degree of development has been attained. The ovaries contain ova which have begun to enlarge, the oviducts and vaginal duct are fused, but are narrow and without a lumen, the colleterial gland is large, but the seminal receptacle is small and vestigial. It is worthy of note that in young colorless soldiers with round heads the size of the ovaries and the contained ova is greater than in adult soldiers, showing that the retrogression of these sex organs does not begin until the soldier is almost adult. On first examining stained whole mounts of these young soldiers the writer was almost convinced that they were fertile, but the explanation may be found in the fact that this species of *Pro-rhinotermes*, and perhaps also the genus, although one of the higher termites, has many primitive characters which link it to the lower termites. The continuation of the period of growth of the soldier sex organs almost to the adult phase, together with the complete fusion of the embryonic fundamentals in both sexes of the two sterile castes; the presence of ocelli in all castes, even in the soldier, as in the *Kalotermitidae*, and especially the presence in the worker of a median bilobed ocellus in the place of the coenogenetic frontal gland, all contribute evidence for this view.

CONCLUSIONS AND SUMMARY

A general study of the termites *Reticulitermes flavipes*, *R. virginicus*, and *Prorhinotermes simplex* establishes the fact of the existence in each of five well-marked types or castes, three reproductive and two sterile, namely: 1) adults of the first form, with long wings; 2) adults of the second form, with wing vestiges; 3) adults of the third form, with no wings; 4) the workers, and, 5) the soldiers.

Throughout these series of forms there is a correlation in the size, structure, and degree of development of the brain, the eyes, and the sex organs; and, in general, there is a gradation in the size of these organs from the first form down to the worker or soldier, this gradation of structure possibly representing different degrees of retrogressive ancestral mutations. On the other hand, certain types possess characters peculiar to themselves, which may represent progressive mutations, for example, the stouter legs of the second forms, the thicker chitin of the head of the workers and soldiers, and the elongated head and mandibles of the latter. The type of venation found in the wings of the first-form adult, namely, the presence of the subcosta, radius, media, and cubitus, is also found in the wing pads of nymphs of the first and second form, and in the wing vestiges of second-form adults, but no trace of the humeral suture is present in either wing pads or wing vestiges.

The enlarged adults of the third form of *Reticulitermes flavipes* have been known since 1915, but the young adults and nymphs are here described for the first time.

In size, general shape, and absence of wings, the nearly mature third-form nymphs and the young third-form adults of *R. flavipes* bear a strong resemblance to workers, but the two castes may be distinguished by the following external characters: color of head, number of antennal segments, size of compound eyes, presence or absence of ocelli, form and color of abdomen. Additional differences are found in internal structures, such as the larger brain and salivary glands, the functional sex organs, the abundant fatty tissue, and the degenerate (adult) jaw muscles

of the third form; in contrast with the smaller brain and salivary glands, the embryonic, non-functional sex organs, the scanty fatty tissue, and the functional jaw muscles of the worker. The dark woody masses in the intestinal sac of the worker and their absence in the third form are evidence of the worker's habit of masticating wood and the probable diet of predigested food in the third form.

The development of third-form nymphs smaller than 4 mm. in length has not been worked out, on account of the difficulty of distinguishing them from worker nymphs of similar age.

The young third-form adults of *Prorhinotermes simplex*, although wingless and similar to workers in size and general shape, are easily recognized by their dark brown body pigment. The young and nearly mature third form nymphs, however, bear a strong superficial resemblance to their workers, but the two castes of this termite may be distinguished by minute points of difference very similar to those which differentiate the third form and worker of *Reticulitermes*.

The venation of the long wings of the first-form adult of *P. simplex* is similar to that of the wing vestiges of the second-form nymph. No second-form adults of this species have yet been described, although the second-form nymphs are very abundant.

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DESCRIPTION OF PLATES

ABBREVIATIONS

<i>br</i> , brain	<i>m</i> , jaw muscles
<i>c</i> , cuticle	<i>oc</i> , ocellus
<i>e.g.</i> , colleterial gland	<i>ov</i> , ovary
<i>f</i> , fat masses	<i>p</i> , penis
<i>f.c.g.</i> , fundament and vestiges of colleterial gland	<i>s.r.</i> , seminal receptacle
<i>f.s.r.</i> , fundament and vestige of seminal receptacle	<i>s.v.</i> , seminal vesicle
	<i>t</i> , testis

PLATE 1

EXPLANATION OF FIGURES

All figures are drawn from semitransparent whole mounts of *Reticulitermes flavipes*. Leitz oc. 1, obj. 3, stage level.

21 Female reproductive organs of a nearly mature nymph of the first form. Only the proximal parts of the ovaries are shown.

22 Female reproductive organs of a nearly mature nymph of the second form. Only the proximal parts of the ovaries are shown.

23 Female reproductive organs of a nearly mature nymph of the third form.

24 Male reproductive organs of a nearly mature nymph of the first form.

25 Male reproductive organs of a mature nymph of the second form.

26 Male reproductive organs of a nearly mature nymph of the third form.

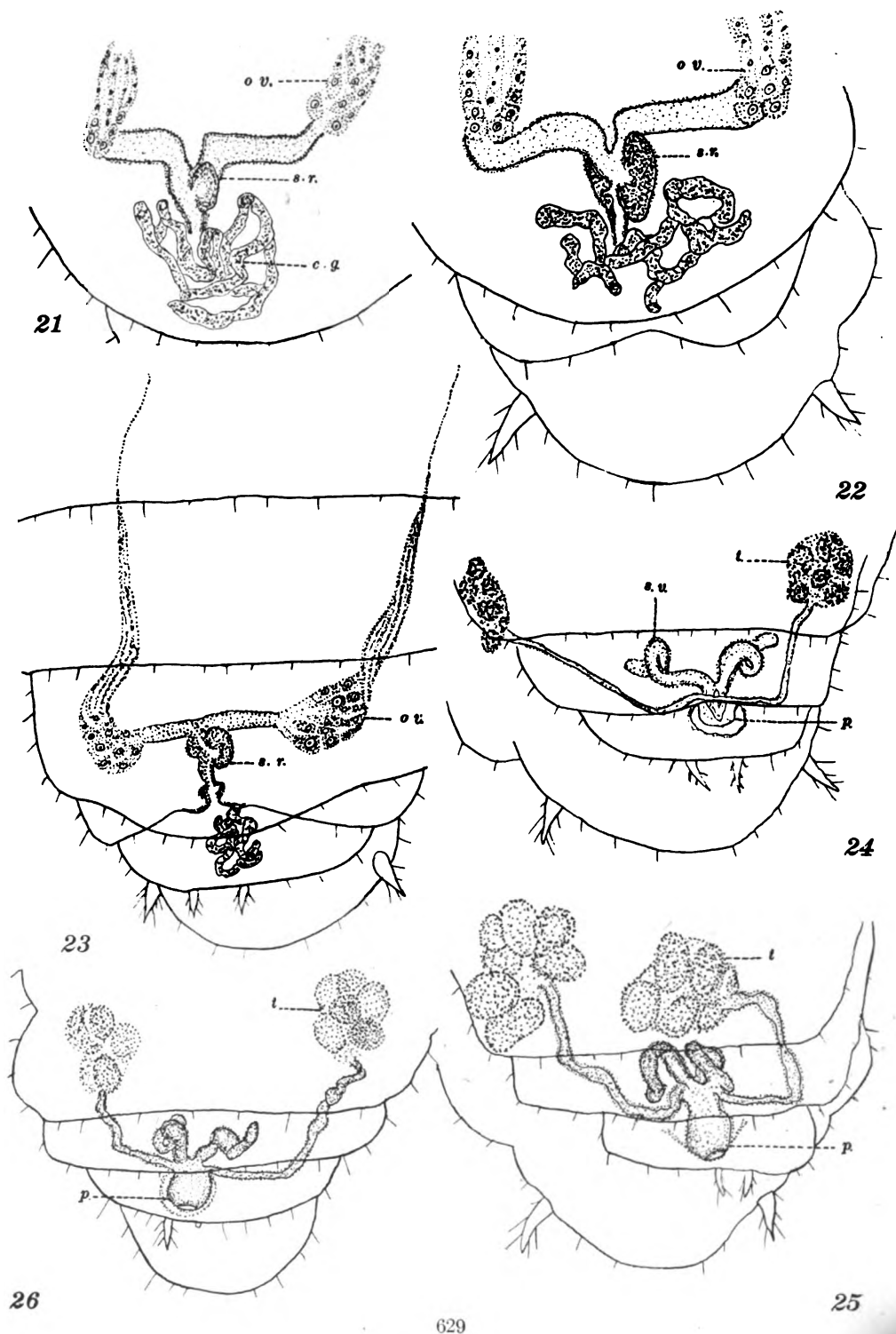


PLATE 2

EXPLANATION OF FIGURES

Figs. 27 to 30 are drawn from semitransparent whole mounts. Leitz oc. 1, obj. 3, stage level. Figs. 31 and 32 are from sections of heads, Spencer oc. 6, obj. 16, stage level. All figures from *Reticulitermes flavipes*.

27 Female reproductive organs of a worker, adult. The three fundamentals of the reproductive system are not fused, either in this caste or in the soldier.

28 Female reproductive organs of an adult soldier.

29 Male reproductive organs of an adult worker.

30 Male reproductive organs of an adult soldier.

31 Section of the head of an adult worker.

32 Section of the head of an adult of the third form.

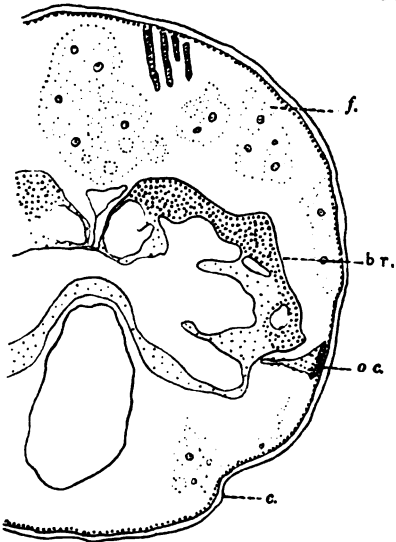
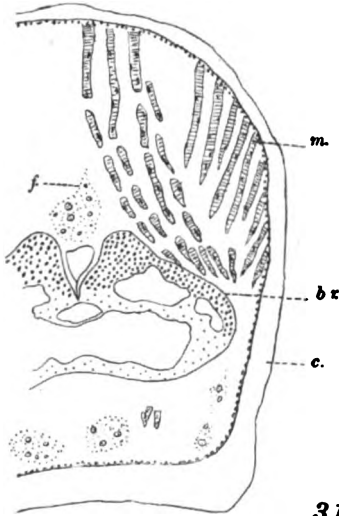
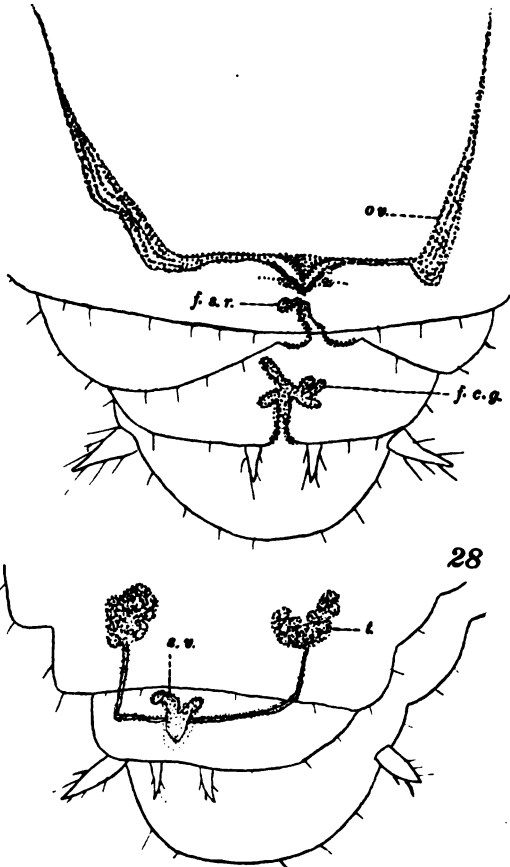
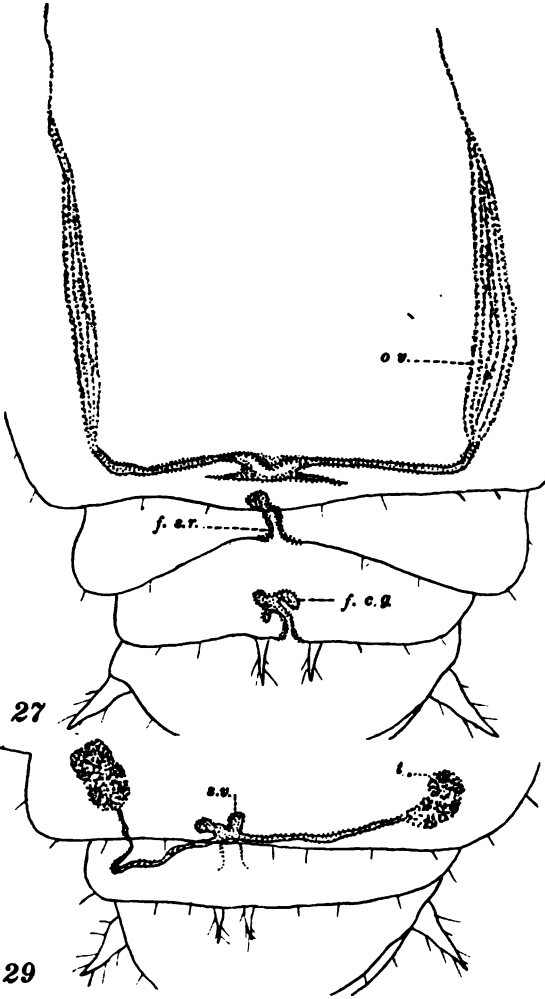
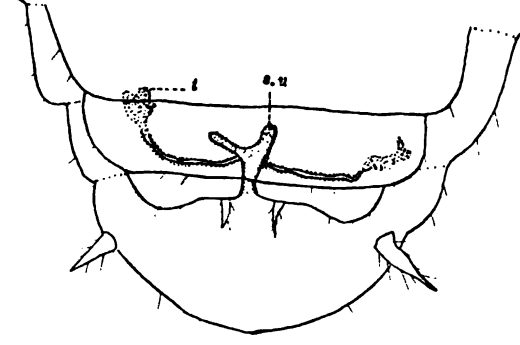
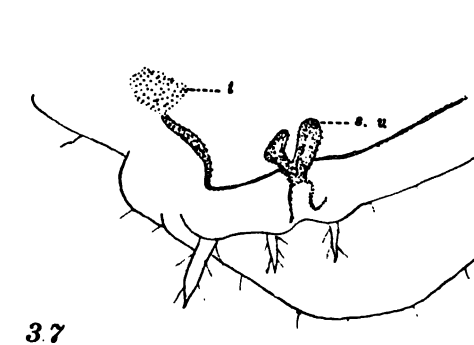
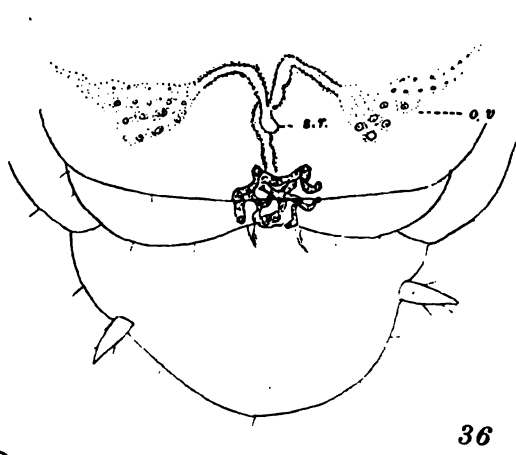
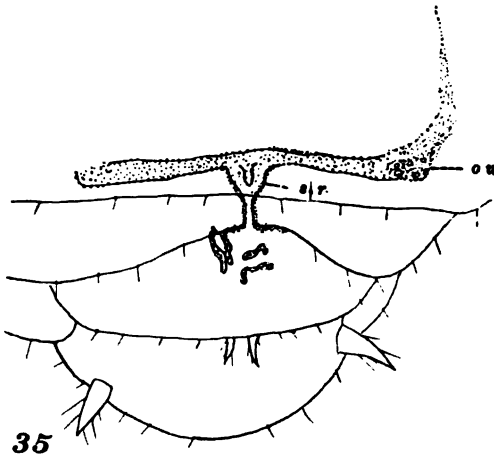
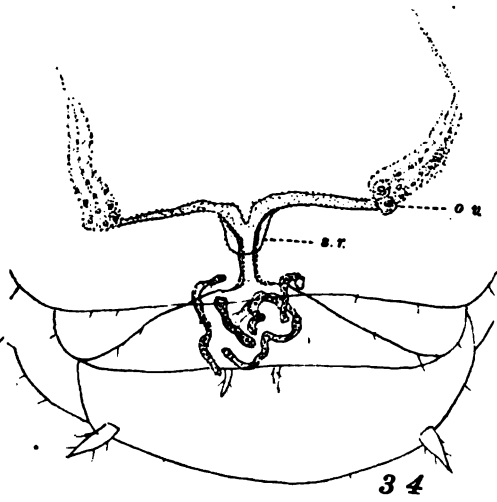
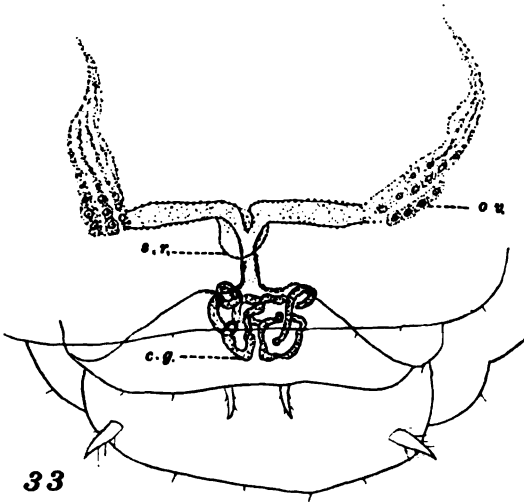


PLATE 3

EXPLANATION OF FIGURES

All figures are drawn from semitransparent whole mounts of *Prorhinotermes simplex*. Leitz. oc. 1, obj. 3, stage level.

- 33 Female reproductive organs of a nymph of the second form.
- 34 Female reproductive organs of a nymph of the third form.
- 35 Female reproductive organs of a worker, adult.
- 36 Female reproductive organs of a soldier, adult.
- 37 Male reproductive organs of a nymph of the second form.
- 38 Male reproductive organs of a soldier, adult.



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